Meta Imaging Series®

MetaFluor

Version 7.0 for

Microsoft Windows XP®

User's Guide

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Navigating Through the MetaFluor Online Help

If you have used a Windows-based online Help system before, you will find the MetaFluor Online Help familiar and easy to use. If you have never used online Help, you should take a moment to read *How to Use Windows Help.* You can do this by positioning the pointer over the highlighted (green) text "How to Use Windows Help" below.

How to Use Windows Help

Most commands listed in MetaFluor's Online Help have three Help pages--a summary page, a procedure page, and a dialog box options page. Summary pages are displayed in a main window. Procedure and dialog box options pages are displayed in Procedure and Dialog Box Options windows, respectively. These are smaller, "secondary" windows. Unlike the main Help window, these two types of secondary window will always stay on top of any other application you are running. This is so that you can read step-by-step procedures or descriptions of dialog box options while you work in MetaFluor.

If you have used a Windows-based online Help file before, you may notice several additional buttons in the MetaFluor Online Help button bar at the top of main window: the *Procedure,* and *Dialog* buttons.

The **Procedure** button displays the command's procedure page.

The **Dialog** button displays the command's dialog options page. Some commands do not have dialog boxes: the *Dialog* button will be disabled for these commands.

Like the main Help window, the Procedure and Dialog Box Options windows have *Procedure* and *Dialog* buttons. You can use these buttons to toggle between the Procedure and Dialog Box Options pages for the **current topic displayed within the smaller window.** To update the contents of the Procedure or Dialog Box Options window with a new topic displayed in the main Help window, choose the *Procedure* or *Dialog* button from the main Help window's toolbar (not the *Procedure* or *Dialog* button in the smaller window).

The Procedure and Dialog Box Options windows also have a *Print* button which allows you to print the current topic in the window. You can also drag the pointer over text that you want to print, click the right mouse button, and choose Print from the shortcut menu that appears.

The **Main** button in the Procedure and Dialog Box Option windows allows you to:

(1) Display the main window if you minimized or closed its window,

(2) Move the smaller window to the left of the main window, or

(3) Close the main window but leave the smaller window open.

There are several other buttons in the button bar of the main Help window which are very useful for navigating in MetaFluor's online Help. The **Browse** buttons **(<<** and **>>)** allow you to browse through topics that are on the same level in the online Help. The **Help Topics** button takes you back to the MetaFluor Help Topics window.

MetaFluor Online Help Structure

Help Topics Dialog Box - Contents Tab

Click the *Contents* tab at the top. You can select an online Help topic by clicking the book or page icon displayed before the topic title. A book icon indicates that there is a group of related topics together. Click the book icon to display these topics. Clicking a page icon will lead you directly to its topic. For example, if you click the File menu icon, you will see a list of its commands. Clicking the Open Experiment page icon will open the summary page for the Open Experiment command.

New Experiment   **Open Experiment**   **. . .**

You can use the Browse buttons to jump between summary pages for commands on this level. You can use the *Procedure* button to jump to "Opening an Experiment" or you can use the *Dialog* button to jump to "Open Experiment - Dialog Box Options."

Opening an Experiment   **Open Experiment - Dialog Box
                                   Options**

You can use the *Procedure* and *Dialog* buttons in the command toolbar of these windows to jump between topics on this level. At this level, the button pertaining to your current page will be disabled unless there are multiple Procedure or Dialog Box Options pages (for complex commands with multiple dialog boxes).

Journal Functions

- A -

Acquire Background
Acquires a background subtraction image.

Acquire Shading
Acquires a shading correction image.

Acquire Stream
Starts stream acquisition of wavelength images as rapidly as possible into computer memory.

Adjust Exposure Time
Adjusts the exposure time for the selected wavelength by increasing or decreasing the exposure time by a selected amount.

Adjust Frames to Average
Adjusts the frame averaging for the selected wavelength by adding or subtracting from the number of frames to average.

Adjust Frames to Integrate
Adjusts the frame integration for the selected wavelength by adding or subtracting from the number of frames to integrate.

Analog Display
Opens or closes analog measurements graphs for a specified analog channel.

Analog Settle Time
Sets how long to wait to acquire an image after changing the analog gain and black level.

Ask to Subtract Backgrounds
Sets whether or not to query the user if background reference images are to be used.

Async Analog Measurements
Starts or stops analog asynchronous measurements.

Auto Shutter
Toggles between an open and closed shutter state when showing live video.

- B -

Beep
Issues the computer's beep sound.

- C -

Channel Settle Time
Sets the analog video channel settle time, in milliseconds, for the specified video channel.

Clear Graph On Reset
Configures whether or not to erase the time-based graphs if the clock is reset during playback.

Clear Message
Clears the message (if any) displayed on the Status window's message line.

Clear Regions

Clears all regions. Closes the Intensity and Ratio graphs if they are open.

Close DIO Driver

Closes the specified DIO (digital I/O) driver.

Close Measurements File

Closes the currently open measurements log file.

Close Save Images File

Closes the .inf file and the associated wavelength image files.

Close Save Ratios File

Clears the selected base name from use in saving a set of ratio images and closes the ratio image files.

Close Serial Driver

Closes the serial driver for the named device.

Configure Background

Configures a background subtraction mode (Image, average gray value in a selected Region, Constant gray value, or None) for each selected wavelength image.

Configure Image Acquisition

Defines the acquisition settings for each wavelength. This includes the Illumination Device control as well as the camera acquisition parameters. You must add a new journal entry for each wavelength whose acquisition parameters you want to define.

Configure Intensifier Gain Control

Specifies the model of ICCD you are using, its serial port, its baud rate, and whether its control is manual or by computer.

Configure Shading

Configures a shading correction mode (Image or None) for each selected wavelength image.

- D -

Delay

Adds a specified amount of time (in milliseconds) to wait before the next command is carried out.

Display Channel Graph

Shows or hides the analog measurements graph for a selected analog data channel.

Display Message

Displays a message on the message line of the Status window.

Display Windows

Opens a selected command's dialog box.

Draw Image Labels

Enables or disables the display of image labels on an external video monitor.

Draw Quadrant Marks

Enables or disables the display of quadrant marks on an external video monitor.

Draw Region Labels

Enables or disables the use of region labels and specifies their placement.

Draw Save Region

Enables or disables the display of the Save Region outline on an external video monitor.

Draw Regions

Enables or disables the display of region of interest outlines on an external video monitor.

- E -

Execute Journal

Runs the specified journal.

- F -

Focus Method

Selects between computer image window and external monitor display for focusing images acquired with a digital camera.

- G -

Graph Channel

Enables or disables graphing of a selected analog measurements data channel.

Graph Clear When Regions Change

Configures whether or not to clear the Intensity and Ratio graphs when regions are changed.

Graph Click Displays Image

Configures whether or not to display the image nearest to the time point in the graphs where the pointer is clicked.

- L -

Load Backgrounds

Loads a reference image for background subtraction.

Load Calibration Standards

Loads a calibration standards file.

Load Event List

Loads a set of event marks from disk.

Load Journal Sequence

Loads a journal sequence file.

Load Journal Toolbar

Loads the specified journal toolbar.

Load Regions

Loads in a set of regions of interest.

Load Shadings

Loads a reference image for shading correction.

Lock Shutter Open

Sets the Lock Shutter Open state.

Log Channel

Enables or disables logging of data from a selected analog measurements channel.

Log Now

Logs the current set of data to an open measurements file.

- M -

Mark an Event in List

Selects the number of an Event Mark List entry in preparation for marking the associated event.

Mark Current Event

Displays the currently selected event mark on the graphs and/or logs it in the open measurements (log) file.

Maximum Graph Points

Selects the maximum number of points to be displayed on time-based graphs.

Measure Channel

Switches asynchronous measurement on or off for a selected analog measurements channel.

Move to Next Event Mark

Moves the highlighter to the next event mark in the Event Mark List.

- O -

One Acquisition Cycle

Performs one cycle of acquisition.

Open DIO Driver

Opens the specified DIO (digital I/O) driver.

Open Experiment Control Panel

Configures whether or not to open the Experiment Control Panel whenever an experiment is opened.

Open Notebook

Configures whether or not to open the Notebook window whenever an experiment is opened.

Open Protocol File

Configures whether or not to open the Load Protocol File dialog box whenever an experiment is opened.

Open Serial Driver

Opens the serial port for the specified device using the specified serial communications parameters.

Open Status

Configures whether or not to open the Status window whenever an experiment is opened.

- P -

Pause Experiment

Pauses a running experiment. This command will not take effect until the journal completes.

Play Sound

Plays a selected sound (*.wav) file (requires a sound card).

- R -

Reset Event Mark Timer

Zeroes the clock used by the Event Marks countdown timer.

Resize Image to Fit Scale Bar

Configures whether or not images will resize to accommodate a scale bar outside of the image area.

Resume Experiment

Resumes running an experiment. This command will not take effect until the journal completes.

Run Journal Sequence

Initiates a journal sequence.

Run Program

Runs an external program from within MetaFluor. You can run the external application in a window that is Normal, Minimized, or Maximized.

- S -

Save Backgrounds

Saves the selected background subtraction image to disk.

Save Channel

Switches binary saving on or off for a selected analog measurements channel.

Save Current Images

Saves the current images.

Save Event List

Saves a set of event marks to disk.

Save Journal Sequence

Saves the journal sequence to a file.

Save Ratio Now

Saves the current ratio image.

Save Settings

Saves the current MetaFluor configuration settings.

Save Shadings

Saves the selected shading correction image to disk.

Scale Bar Continuous

Configures whether to make the scale bar continuous or discrete.

Scale Bar Drawing

Configures whether or not to draw scale bars, and if so on what images to draw them.

Scale Bar Location

Sets the location for the scale bar.

Scale Bar Stamp

Configures whether or not to stamp the scale bar on images.

Send DIO Data

Sends DIO (digital I/O) data using the selected DIO driver.

Send Serial Data

Sends the specified serial command string to the named device. The Data to Send is based on the device's documentation.

Set Analog Settle Time

Sets the analog settle time, in milliseconds.

Set Analog Values

Sets the analog black level and white level for the selected video channel.

Set Bit Depth

Sets the digital camera bit-depth to use when acquiring a specific wavelength.

Set Camera Black Level

Sets a video camera's black level.

Set Camera Shutter

Sets the shutter's state for a selected wavelength image.

Set Camera Video Gain

Sets a video camera's gain.

Set Correct Shading

Enables or disables shading correction.

Set Condition

Defines up to five experimental conditions which can be used to "tag" the experimental data at appropriate times.

Set Display Mode

Sets the display mode for a wavelength, ratio, or quadrant display on an external video monitor.

Set Exposure Time

Sets the exposure time for digital cameras to the specified number of milliseconds, seconds, or minutes.

Set Frames to Average

Sets the frame averaging time for the Matrox Image-LC board to the specified number of frames. Use the value "1" for no frame averaging.

Set Gain

Sets the digital camera gain for acquisition of a selected wavelength image.

Set Image Update

Enables or disables image updating for the selected image.

Set Image Update Interval

Sets the image updating interval for the selected image.

Set Integration Time

Sets the number of frames to average for images of a specified wavelength.

Set Intensifier Gain

Sets the gain of the ICCD's intensifier.

Set Intensity

Directly changes the Intensity setting of the desired Illumination MetaDevice.

Set Log Data

Enables or disables data logging to the open measurements (log) file.

Set Number of Acquisitions

Sets the acquisitions acquired to the specified number.

Set Ratio Display

Configures the display mode and ratio range for a selected ratio image.

Set Ratio Name

Specifies a name for a selected ratio image.

Set Save Calibration Map

Configures whether or not to save Calibration Maps.

Set Save Calibration Map Sequence

Sets the sequence name for saving Calibration Maps.

Set Save Images

Enables or disables wavelength image saving.

Set Save Interval

Specifies a saving interval for a selected wavelength or ratio image.

Set Save Ratios

Enables or disables ratio image saving.

Set Shading

Enables or disables shading correction.

Set Shutter

Directly changes the Shutter state of the desired Illumination MetaDevice.

Set Speed

Sets the digital camera transfer speed for acquisition of a selected wavelength image.

Set Subtract Backgrounds

Enables or disables background subtraction.

Set Thresholds

Sets the high and low threshold limits for images of a selected wavelength image.

Set Timelapse

Sets the timelapse interval to the specified number of milliseconds, seconds, minutes, or hours.

Set Wave 1 Intensifier Gain

Sets the gain of the ICCD intensifier for the Wavelength 1 image.

Set Wave 2 Intensifier Gain

Sets the gain of the ICCD intensifier for the Wavelength 2 image.

Set Wave 3 Intensifier Gain

Sets the gain of the ICCD intensifier for the Wavelength 3 image.

Set Wavelength

Directly changes the Wavelength setting of the desired Illumination MetaDevice.

Set Wavelength Acquisition

Enables or disables acquisition for a selected wavelength image.

Set Wavelength Acquisition Interval

Sets the acquisition interval for a selected wavelength image.

Set Wavelength Display

Configures the display mode, brightness and contrast, threshold levels, and 16-bit scaling for a selected wavelength image.

Set Wavelength Name

Specifies a name for the display window of a selected wavelength image.

Show Dialog on Event

Configures whether or not to display the associated dialog when an event mark occurs during playback.

Show Event List on Playback

Configures whether or not to open the Event List whenever an experiment is opened.

Show Live

Stops running an experiment and shows live video. This command will not take effect until the journal has finished.

Show Message and Wait

Displays the selected message in a dialog box and waits for the specified number of seconds for the user to choose OK or Cancel.

Show or Hide Image Window

Configures whether or not to display a selected wavelength or ratio image.

Stop Playing Sounds

Terminates the sound (*.wav) file currently being played.

Subtract Backgrounds From Loaded Calibration Image

Sets whether or not to subtract a background image when calibration reference images are loaded.

Summarize Regions

Configures whether or not to average all regions of the same color during measurement procedures.

- T -

Transfer Regions

Transfers regions from the active image to another specified image.

Twain Configure

Selects a TWAIN-compliant device for image acquisition and specifies whether to use the device's user interface.

- U -

Use Auto-Execute Journals

Sets whether or not to use Auto-Execute Journals.

Use Channel

Enables or disables use of a selected analog measurements channel.

Use Frame Averaging

Sets whether or not to use frame averaging when showing live video.

Use Graph Markers

Configures whether or not to use graph markers.

Use Same Analog Settings for All Wavelengths

Sets whether or not to use the same analog contrast settings for all wavelength images.

Use Sequence Journals

Sets whether or not to use Sequence Journals.

Use Trigger Journals

Sets whether or not to use Trigger Journals.

- W -

Wait for DIO Data

Waits for DIO (digital I/O) data to be received from a specified DIO driver.

Wait for Serial Data

Waits for serial data to be received from a specified serial driver.

Wait for Trigger

Waits for one of the Trigger Journals conditions to be met.

Write to Log File

Logs text you enter in the Text to Log text box to an open measurements file.

- Z -

Zero Clock

Resets the clock to zero.

Zero Cycle Count

Resets the cycle counter to zero.

Zero Sequence Clock

Resets the sequence clock to zero.

Zero Sequence Counter

Resets the sequence counter to zero.

Zero Timelapse Countdown

Resets the timelapse counter to zero.

File Menu

New Experiment (File Menu)

> **Prepares MetaFluor for a new experiment by opening an image window for each selected wavelength and/or ratio that you have defined for your experiment and opening the Experiment Control Panel.**

Use this command when you want to begin acquiring new data. When you start MetaFluor, most of its commands will be unavailable and will appear dimmed until you choose New Experiment or Open Experiment. The appropriate commands and image display will appear after you choose New Experiment. If you want to play back a saved experiment, use the Open Experiment command instead of the New Experiment command.

Note: This command is unavailable in the MetaFluor Offline system.

For most camera and board configurations, your computer monitor will display acquired images from a new experiment in image windows named Wavelength 1, Wavelength 2, Ratio 1, Wavelength 3, etc. These image windows will open automatically when you choose the New Experiment command.

If you want, you can configure MetaFluor to prompt you for an appropriate protocol file by selecting *Prompt to Select a Protocol File* in the General Preferences dialog box (Preferences command, File menu).

Depending on the preferences you set using the **Preferences** command, the **Notebook** and the **Status window** may also appear when you start a new experiment.

> **See Also:**

Open Experiment

Close Experiment

Open Status Window

Open Notebook

Load Protocol File

Preferences

Starting a New Experiment

To start a new experiment, use the following procedure:

Step	Action
1	From the File menu, choose New Experiment.
2	Wait while MetaFluor starts the new experiment, loads a protocol file, and opens the Status window and the Notebook (if so configured). If a journal toolbar was open when the protocol file was last saved, it will be opened, too.
3	Image windows will appear on your computer monitor.

Open Experiment (File Menu)

Opens a previously acquired experiment that was stored on the hard disk so that you can play back and examine its images.

Use this command when you want to play back and examine images from an experiment that has been stored on the hard drive. When you start MetaFluor, most of its commands will be unavailable and will appear dimmed until you choose Open Experiment or New Experiment. The appropriate commands and image display will appear after you choose Open Experiment. If you want to start a new experiment, you should use the New Experiment command, rather than the Open Experiment command.

An experiment can be played back only if the images acquired during the experiment were saved to disk. MetaFluor does not have a "Save Experiment" command. Rather, it allows you to specify base file names for saving sets of wavelength and/or ratio images, and direct MetaFluor to save images during the experiment by selecting or clearing the appropriate check box in the Experiment Control Panel.

When you open an experiment, MetaFluor will build the ratio images using the saved wavelength image pairs. This allows you to conserve disk space because you will not be required to save the ratio images. (You can, however, save ratio images either during the acquisition or during playback. You may wish to do so to build a movie from them or to load them into MetaFluor.)

Your computer will load the saved images into image windows named Wavelength 1, Wavelength 2, Ratio 1, Wavelength 3, etc.

When you want to open an experiment that uses settings different from those used in your last session, you may first need to load the pertinent protocol file that was active during acquisition. Otherwise, some options may be different or missing during playback. You can configure MetaFluor to prompt you for the appropriate protocol file by selecting *Prompt to Select a Protocol File* in the General Preferences dialog box (Preferences command, File menu).

See Also:

New Experiment

Close Experiment

Load Protocol File

Preferences

Opening an Experiment

To open an experiment, use the following procedure. (If necessary, you should load the appropriate protocol file.)

Step	Action
1	From the File menu, choose Open Experiment. The Select Image File (INF File) dialog box will appear.
2	Select the desired .inf file. If necessary, use the *Look In* list or the Up One Level button to select the appropriate drive and folder.
3	Choose *Open.*
4	MetaFluor will open the experiment's images and build ratio images from each pair of images.

Open Experiment - Dialog Box Options

File Name

Lists the name of the currently selected file.

Files of Type

Determines the file format of the files displayed in the *File Name* list. Select *All Files (*.*)* to display all file names.

Look In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

Open

Opens the experiment.

Cancel

Cancels the command.

Close Experiment (File Menu)

Closes the current experiment.

Use this command when you want to close the current experiment. You must close the current experiment to start a new experiment or play back another one. If any files are open, they will be closed when the experiment is closed.

See Also:

New Experiment

Open Experiment

Closing an Experiment

To close an experiment, use the following procedure:

Step	Action
1	Select the File menu.
2	Choose Close Experiment.
3	A dialog box will appear, asking if you want to save a custom protocol file while closing the experiment. Choose:

Yes to close the experiment and save the protocol file,

No to close the experiment without saving the protocol file, or

Cancel to cancel the Close Experiment command.

Get Info (File Menu)

Displays configuration and acquisition information for the current experiment.

Use this command when you want to view information about the current experiment, such as the timelapse interval, exposure time, camera gain, and illumination settings. An annotation text box and a table which lists the current set of event marks are also available in this dialog box. You can export the information to a text file, copy it to the Clipboard, or send it to a printer. Choosing this command will display much of the same information as is displayed for the Load Protocol File or Save Protocol File commands, with the exception that the commands for choosing the file and directory will be absent.

Shortcut: ALT + I

See Also:

Load Protocol File

Save Protocol File

Event Marks

Getting Information About an Experiment

To view information about an experiment, use the following procedure:

Step	Action
1	From the File menu, choose Get Info. The Get Info dialog box will appear.
2	From the *Wavelength* drop-down list, select the wavelength for which you want to see the configuration, acquisition, and display settings.
3	The *Protocol Annotation* text box will show any previously stored comments for the file that you may have entered from the Save Protocol dialog box's *Description* text box. You can enter an annotation or edit the existing one.
4	If you want to print or export the information in the Get Info dialog box, choose *Export*. The Export dialog box will appear. Choose
	Save Info to a Text File if you want to store the information as a text file. The export Info to File dialog box will appear. Type a name for the file in the *File Name* text box, use the *Save In* list or Up One Level button to select the drive and folder if necessary, and choose *Save.*
	Copy Info to Clipboard if you want to copy the information to the Clipboard for pasting into another Windows-based program.
	Print Info if you want to send the information to a printer.
5	Repeat Steps 2 - 4, as necessary, for any other wavelengths.
6	When you have finished, choose *Close.*

Get Info - Dialog Box Options

Protocol Annotation

This editable text box displays any comments that were entered when the current protocol file was last saved. When you type a new annotation or edit the existing one, you can press CTRL + ENTER to skip to the next line in the annotation.

Timelapse Interval

Displays the time interval between image acquisitions. If images were not acquired in timelapse fashion, this will read "0 sec."

Load INF

Displays the path for the folder from which the .inf file will be loaded.

Save INF

Displays the path for the folder where the .inf file will be saved.

Acquire Image

Specifies whether image acquisition has been enabled.

Update Image

Specifies whether updating of image display has been enabled.

Display Window

Specifies whether the image will be displayed.

Save Image

Specifies whether image saving has been enabled.

Exposure Time

Displays the exposure time for the selected wavelength image (digital acquisition).

Camera Gain

Displays the gain setting if your digital camera supports this feature.

Illum. Device

Displays the name of the installed Illumination MetaDevice used for the selected wavelength image.

Wavelength

Displays the wavelength of illumination for the selected wavelength image.

Intensity

Displays the intensity of illumination for the selected wavelength image.

Use Shutter

Displays the shutter usage status *(Yes* or *No)* for the selected wavelength image.

Wavelength

Selects the wavelength image for which the configuration, acquisition, and display information is displayed. Most of these settings were specified in the Configure Acquisition dialog box (Configure menu).

Event Marks

Displays the event marks in the current *Events List.*

Export

Opens the Export dialog box, from which you can save the information to a text file, copy it to the Clipboard, or send it to a printer.

Close

Closes the dialog box.

Open Measurements File (File Menu)

Opens the measurements (log) file for storing measurements made during an experiment.

Use this command to open a measurements file before logging measurement data from an experiment. Measurement data collected from either new or saved experiments can be logged to a text file, logged directly to an open spreadsheet program by Dynamic Data Exchange (DDE), or both. These files, also called log files, allow you to store measurement data on disk so that you can view the data after you complete an experiment.

To log measurement data, MetaFluor must know *where* you want the data stored, that is, which text file or spreadsheet application to use. This information is supplied by the Open Measurements File command. *What* is logged is determined by the types of measurement data you have selected using the **Configure Experiment** command. However, even if a log file is open and configured, nothing will be logged until you select the *Log Data* check box in the Experiment Control Panel (Run Experiment menu). This option allows you to log measurement and event data selectively *when* you need it.

If you move or redefine regions with the Define Regions for Measurement command while saving regional data to a measurements file, and want to log the new region information automatically (new coordinates, size, and thresholded area), you can do so by selecting the *Log Header After Editing Regions* check box in the Data Logging Preferences dialog box (Preferences command, File menu). This will *not* be the case when regions are moved with the Move All Regions command, however, because a large number of meaningless log entries would be generated during the movement and resizing procedures.

See Also:

Preferences

Experiment Control Panel:

For Video Camera with External Monitor

For Video Camera with Computer's Monitor

For Digital Camera

Close Measurements File

Define Regions for Measurement

Opening a Measurements File

To open a measurements file, use the following procedure:

Step	Action
1	Start a new experiment or open a stored experiment from which you want to log measurement data.
2	From the File menu, choose Open Measurements File. The Measurements dialog box will appear.
3	Select where you want the data to be logged:
	To an open spreadsheet program by *Dynamic Data Exchange (DDE),*
	To *A Text File,* or
	To both.
4	If you selected *A Text File* in the previous step, the Open Measurement Log File dialog box will appear.
	If necessary, select the destination drive and folder for the log file using the *Save In* list or Up One Level button. Type the desired file name in the *File Name* text box.
	AND
	Choose *Save* to close the dialog box.
5	If you chose an existing log file name in the previous step, the Log File Exists dialog box will appear. You can *Overwrite* the contents of the file, *Append* new data to the file, or *Cancel* the command.
	Note: If you choose *Cancel,* you can specify a different file name by repeating Steps 2 - 4.
6	If you selected *Dynamic Data Exchange (DDE)* in Step 3, the Export Log Data dialog box will appear.
7	Select the desired application from the *Application* drop-down list. Choose *Default* to use the default settings for that application.
	AND
	Choose *OK.*
	Note: The spreadsheet application must be open before you can create a DDE link to a worksheet.
8	The status line for *Log Data* in the Experiment Control Panel will now indicate that you have opened a log file or activated a DDE link.

Open Measurements Log File - Dialog Box Options

File Name

Lists the name of the currently selected file.

Files of Type

Determines the file format of the files displayed in the *File Name* list. For opening log files, the default is *.LOG.*

Save In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

Save

Opens the log file.

Cancel

Cancels the command.

Creating a DDE Link to Excel

Using a New Microsoft Excel Worksheet

If you already have data in an open spreadsheet, you can request that MetaFluor link to a new Microsoft Excel worksheet. After you have selected the appropriate program version from the *Application* drop-down list in the Export Log Data dialog box, type <NEW> in the *Sheet Name* text box (include the brackets surrounding the word "New"). This will open the next new sheet--Book2[Sheet1] (or Sheet2 in Microsoft Excel 4.0).

Using a Microsoft Excel Worksheet Other Than the Default Worksheet

To use a previously saved worksheet, type its path and file name in the *Sheet Name* text box of the Export Log Data dialog box. For example, type the following in the *Sheet Name* text box:

    C:\MSOFFICE\EXCEL\SHEETS\TEST.XLS

Then carefully select the *Starting Row* and *Starting Column* values for the new data so that you do not use rows and columns that already contain data. After you have done this, you can choose *OK* to close the Export Log Data dialog box.

If you selected the wrong file name for the sheet, the Connect to Application dialog box will appear. Select the correct name from the list at the bottom of the dialog box. Or you can create a new sheet by selecting *<New>.* Then choose *Connect.*

Creating a DDE Link to Lotus 1-2-3, Borland Quattro Pro, or MicroCal Origin

Creating a DDE link to the default worksheet in Lotus 1-2-3 for Windows, MicroCal Origin, or Borland Quattro Pro is similar to the procedure for Microsoft Excel, except that you will need to select the appropriate spreadsheet application name from the *Application* drop-down list. In this case, the default *Sheet Name* is *Untitled.*

If you want to use a previously saved worksheet, you must both start the spreadsheet program and open the worksheet prior to using the Open Measurements File command. Type the path and file name of the worksheet in the *Sheet Name* text box in the Export Log Data dialog box (for example, type: C:\123R4W\SHEETS\TEST.WK4). Then carefully select the starting row and column for the new data so that you do not use rows and columns that already contain data.

If you select the wrong name, the Connect to Application dialog box will appear. Select the correct name from the list at the bottom of the dialog box and choose *Connect.* The *<New>* option for creating a new sheet is not available for Lotus 1-2-3.

Linking to Another Application

You can use an application for DDE that is not listed in the *Application* drop-down list in the Export Log dialog box.

To do so, select *Other Application* from the *Application* drop-down list in the Export Log Data dialog box. You must supply the information for the *Application Name, Topic Name, Item Name, Starting Row,* and *Starting Column.* You will need to contact the software developer's technical support department or consult its user's manual to determine the first three options. You will need to determine the numbers used for the *Starting Row* and *Starting Column.*

Option	Description	Example
Application Name	Defined by the application receiving the data. Typically it is a single word that refers to the software.	EXCEL
Topic Name	Defined by the application receiving the data. For spreadsheets, it is the name of the worksheet that will receive the data.	Sheet1 [Book]Sheet1
Item Name	Defined by the application receiving the data. Specifies where the data are to be sent. MetaFluor recognizes two special symbols in this text string: "<r>" which is replaced by the current row number/letter and "<c>" which is replaced by the current column number/letter.	Application's Format: R1C1 RAC1 Enter in MetaFluor: R<r>C<c>

Measurements - Dialog Box Options

Log Measurements To

Specifies whether the measurements will be logged to an open spreadsheet program by *Dynamic Data Exchange (DDE),* logged to *A Text File,* or both. Select both check boxes if you want to log data to both an open spreadsheet program and a text file.

OK

Opens the Export Log Data dialog box so that you can select an open, DDE-linked spreadsheet program. Opens the Open Measurement Log File dialog box so you can specify a text file, depending on the choice(s) selected for *Log Measurements To.*

Cancel

Cancels the command.

View Measurements (File Menu)

Displays the contents of a measurements file in table format within the Viewer window.

Use this command to view the current log file or another log file in the Viewer window. You can view more than one log file at a time: MetaFluor will open separate viewers for each file. This command only displays the contents of text-based log files; you must use the spreadsheet program to view data that were logged by a DDE link.

The viewer allows you only to view the data stored in the log file. You will not be able to edit or add information to the log file using the viewer.

See Also:

Open Measurements File

Close Measurements File

Viewing Measurements

To view a log file while in image acquisition or playback mode, use the following procedure. (If you are not in image acquisition or playback modes, follow this procedure using the option for *Another Log File.*

Step	Action
1	From the File menu, choose View Measurements. A secondary menu will appear.
2	From the secondary menu, choose:
	Current Log File to view the log file that is currently open, or
	Another Log File to view a log file other than the current log file. You must use this option if a log file is not currently open.
3	If you chose Another Log File in the previous step, the Select Measurement Log File dialog box will appear.
	Select the desired log file. If necessary, use the *Look In* list or the Up One Level button to select the appropriate drive and folder. Choose *Open.* MetaFluor will display the contents of the selected log file in the Viewer window.

Select Measurement Log File - Dialog Box Options

File Name

Lists the name of the currently selected file.

Files of Type

Determines the file format of the files displayed in the *File Name* list. For viewing log files, the default is **.LOG.* Select *All Files (*.*)* to display all file names.

Look In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

Open

Opens the measurements log file.

Cancel

Cancels the command.

Close Measurements File (File Menu)

Closes the active text-based measurements file and/or closes the DDE link to the open spreadsheet program. This command also clears the *Log Data* check box in the Experiment Control Panel if it has been selected.

Use this command to close the measurements file and/or the DDE link to the spreadsheet program when you have finished logging measurements from an experiment. If the *Log Data* check box has been selected in the Experiment Control Panel dialog box (Run Experiment menu), it will be cleared automatically when the log file or DDE link is closed.

Note: This command only closes the DDE link to the open worksheet. You will still need to switch to the spreadsheet program to save the worksheet and quit the program.

See Also:

Experiment Control Panel:

    For Video Camera with External Monitor

    For Video Camera with Computer's Monitor

    For Digital Camera

Open Measurements File

Closing a Measurements File

To close a measurements file, use the following procedure:

Step	Action
1	Select the File menu.
2	Choose Close Measurements File.
3	MetaFluor will clear the *Log Data* check box in the Experiment Control Panel if it has been selected. The status line next to *Log Data* will change from "DDE" and/or "*Filename*.log " to "[File not open]." MetaFluor will close the DDE link to the spreadsheet program if you are logging by DDE.
	Note: This command only closes the DDE link to the open worksheet. You will still need to go to the spreadsheet program to save the worksheet and quit the program.

Open Save Images File (File Menu)

Opens an existing experimental information (*.inf) file, or creates a new one, for the purpose of saving images.

Use this command when you want to save newly acquired wavelength images during an experiment.

To save images, MetaFluor must know *where* you want the data stored, that is, which image files to use or create when it stores the images. This information is supplied to the .inf file by the Open Save Images File command. *What* is saved is determined by whether you have instructed MetaFluor to save the entire image or only a region of the image using the Select Save Region command. However, even if an .inf file is open and saving has been configured, nothing will be saved until you select the *Save Images* check box in the Experiment Control Panel (Run Experiment menu). This option allows you to save wavelength images selectively *when* you need them. "LED" indicators next to the *Save Images* check box will indicate the saving status of each Wavelength image: gray indicates that saving is off, red indicates that saving is on but that the particular Wavelength image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle.

The file name that you specify in the Open Save Images File dialog box will be used in the name for a special file that MetaFluor uses to save a list of the image pairs and their file names, timestamps, and event marks for the series. MetaFluor will add the letter "d" to the end of the name and assigns it the extension ".inf." When MetaFluor saves the corresponding images, it will add the number 1 with a sequentially numbered extension for Wavelength 1 images. File names for Wavelength 2 images will end with the number 2 and the same sequentially numbered extension, file names for Wavelength 3 will end with 3, and so on.

INF File	Wavelength 1 Files	Wavelength 2 Files
ExperD.INF - list of image pairs and file names - time stamps - event marks	**Exper1.001** **Exper1.002** **Exper1.003**	**Exper2.001** **Exper2.002** **Exper2.003**
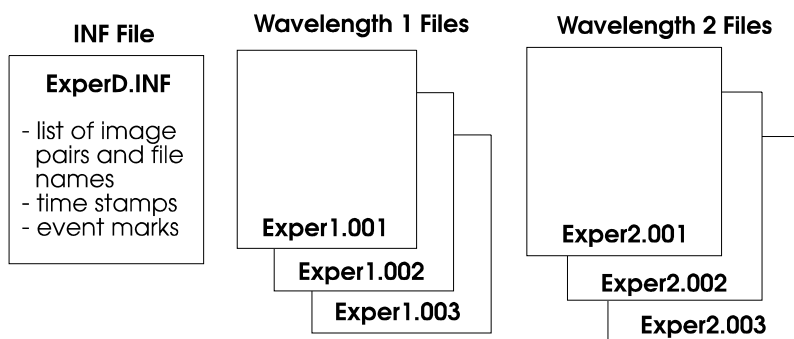
QUICK TIP: A quick way to invoke the Open Save Images File command is to select *Save Images* in the Experiment Control Panel. MetaFluor will open the Save Images File (INF File) dialog box so that you can select a base file name and open an experimental information (*.inf) file. When you have finished, *Save Images* will be selected.

See Also:

Close Save Images File

Experiment Control Panel:

   For Video Camera with External Monitor

   For Video Camera with Computer's Monitor

   For Digital Camera

Saving Wavelength Image Files

To open an information (*.inf) file for saving wavelength images, use the following procedure:

Step	Action
1	Start a new experiment or open a stored experiment from which you want to save images. Load a protocol file if necessary.
2	From the File menu, choose Open Save Images File. The Save Images File (INF File) dialog box will appear.
3	Type a new base file name for the wavelength images in the *File Name* text box, or select the icon for the desired .inf file if you want to use an existing file name. If necessary, use the *Save In* list or the Up One Level button to select the appropriate drive and folder. Then select the file name. **AND** Choose *Save* to close the Save Images File (INF File) dialog box.
4	If you chose an existing file name, the File Exists dialog box will inform you that the file already exists. MetaFluor will ask you how you want to handle the previous contents of the selected file. You can *Overwrite* the contents of the file, *Append* new data to the file, or *Cancel* the Open Save Images File command.
5	If you selected *Overwrite* in Step 4, another message box will appear, asking you to verify that you want to overwrite. Choose *Yes* if you want to do so.
6	The status line for *Save Images* in the Experiment Control Panel will now indicate the name of the open .inf file.

Save Images File (INF File) - Dialog Box Options

File Name

Lists the name of the currently selected .inf file, or specifies a new one, which will be used as the base file name for the wavelength (intensity) images.

Files of Type

Determines the file format of the files displayed in the *File Name* list.

Save In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

Save

Opens the experimental information (*.inf) file.

Cancel

Cancels the command.

Close Save Images File (File Menu)

Closes the .inf file and the associated wavelength image files.

Use this command to close the Information (*.inf) file and associated wavelength image files when you have finished saving images from an experiment. This command also clears the *Save Images* check box in the Experiment Control Panel (Run Experiment menu) if it has been selected.

See Also:

Experiment Control Panel:

For Video Camera with External Monitor

For Video Camera with Computer's Monitor

For Digital Camera

Open Save Images File

Terminating Wavelength Image Saving

To close an .inf file after saving wavelength images, use the following procedure:

Step	Action
1	Select the File menu.
2	Choose Close Save Images File.
3	MetaFluor will clear the *Save Images* check box in the Experiment Control Panel if it is still selected. The status line next to *Save Images* will change from "*FILENAME*.INF" to "[File not open]."

Open Save Ratios File (File Menu)

Selects a base name for an existing set of ratio images that you want to open, or selects a new name for the purpose of saving ratio images.

Use this command when you want to save ratio images during the acquisition of new images or during playback of an existing experiment.

Most of the time, you probably will not want to save ratio images because MetaFluor can build ratio images from the wavelength images whenever you open an experiment. However, if you want to create a movie from the ratios or export the ratios to MetaFluor for further analysis, you must save the ratio images first.

Each ratio image is saved as a .tif file using a sequential number format consisting of a unique sequentially numbered name. The letters "A" and "B" are used to distinguish ratio A (Wavelength 1 / Wavelength 2) from ratio B (Wavelength 4 / Wavelength 5). For example, a series of ratio images might consist of the following files: RatioA0001.tif, RatioB0001.tif, RatioA0002.tif, RatioB0002.tif, RatioA0003.tif, etc. MetaFluor will add the four digits to the end of the name, to signify the acquisition cycle number.

After you have selected a base name for the set of ratio images, you can save the ratio images by selecting the *Save Ratios* check box in the Experiment Control Panel (Run Experiment menu) as needed.

QUICK TIP: A quick way to invoke the Open Save Ratios File command is to select *Save Ratios* in the Experiment Control Panel dialog box. MetaFluor will open the Open Save Ratios File dialog box so you can select a base name for the ratio image set. When you have finished, *Save Ratios* will be selected in the Experiment Control Panel. "LED" indicators next to the *Save Ratios* check box will indicate the saving status of each Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle.

See Also:

Experiment Control Panel:

For Video Camera with External Monitor

For Video Camera with Computer's Monitor

For Digital Camera

Close Save Ratios File

Saving Ratio Image Files

To carry out the Open Save Ratios File command, use the following procedure:

Step	Action
1	Start a new experiment or open a stored experiment from which you want to save images. Load a protocol file if necessary.
2	From the File menu, choose Open Save Ratios File. The Save Ratios File dialog box will appear.
3	Type the base name for the ratio image series in the *File Name* text box. MetaFluor will add four digits and assign the extension ".tif" to your name. Choose *OK.* **WARNING:** MetaFluor will automatically continue the naming sequence that you select for the first image--you must verify that there are no other series of ratio images that use this sequence before you select a name.
4	If you selected a file name that already exists, MetaFluor will display a warning dialog box. Choose *Yes* if you want to replace the existing files. **OR** If you want to select another file name, choose *No.*
5	The status line for *Save Ratios* in the Experiment Control Panel will now display the base name of the ratio image series.

Open Save Ratios - Dialog Box Options

File Name

Lists the name of the currently selected file or specifies a new one, which will be used as the base file name for the ratio image series.

Files of Type

Determines the file format of the files displayed in the *File Name* list.

Save In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

Save

Opens the first image in the selected ratio image series, or saves the selected base name for a new set of ratio images.

Cancel

Cancels the command.

Close Save Ratios File (File Menu)

Clears the selected base name from use in saving a set of ratio images.

Use this command when you have finished saving ratio images from an experiment. This command also clears the *Save Ratios* check box in the Experiment Control Panel (Run Experiment menu) if it has been selected.

See Also:

Experiment Control Panel:

For Video Camera with External Monitor

For Video Camera with Computer's Monitor

For Digital Camera

Open Save Ratios File

Terminating Ratio Image Saving

When you have finished saving ratio images, use the following procedure:

Step	Action
1	Select the File menu.
2	Choose Close Save Ratios File.
3	MetaFluor will clear the *Save Ratios* check box in the Experiment Control Panel if it is still selected. The status line next to *Save Ratios* will change from "*FILENAME*" to "[File not open]."

Load Protocol File (File Menu)

Loads a protocol file for use during an experiment.

Use this command to load a protocol file that was saved previously with the Save Protocol File command.

A protocol file stores each dialog box's selected options and position on-screen. Saving a protocol file for each type of experiment (ratio, single-wavelength, and Delta F/F) will eliminate the need to reconfigure such options as data logging or acquisition and display each time you complete an experiment. MetaFluor protocol files use the extension ".fsf".

If you want to be prompted to load a protocol file whenever you open an experiment or start a new one, you can configure an option in the General Preferences dialog box to do so (Preferences command, File menu).

The settings for the currently active protocol file can be viewed at any time using the Get Info command. The Get Info command can save the protocol settings to a text file, copy them to the Clipboard, or send them to a printer.

See Also:

Preferences

Save Protocol File

Get Info

Loading a Protocol File

To open a protocol file for a particular experiment, use the following procedure:

Step	Action
1	From the File menu, choose Load Protocol File. The Load Protocol dialog box will appear.
2	Choose *Select Protocol Directory.* The Select Directory dialog box will appear.
3	Open the desired folder. If necessary, use the *Save In* list or Up One Level button to select the desired folder. Then select any file in the folder and choose *Select.* The Select Directory dialog box will close, and the path for the selected directory will appear in the Load Protocol dialog box's *Protocol Files Are Located In* status line.
4	Select the desired protocol file from the list by clicking its entry in the *Protocol Files (*.FSF)* list table.
5	Choose *OK.* The selected protocol file be loaded, and the Load Protocol dialog box will close.

Load Protocol - Dialog Box Options

Select Protocol Directory

Opens the Select Directory dialog box, from which the current directory can be changed. After you select the directory, its path will appear in the *Protocol Files Are Located In* status line.

Protocol Files (*.FSF)

Selects one of the protocol files in the current folder.

Protocol Files Are Located In

Indicates the current directory, as selected with the *Select Protocol Directory* command button.

Description

Displays any annotation that was entered when the protocol file was saved.

Timelapse Interval

Displays the time interval between image acquisitions. If images were not acquired in timelapse fashion, this will read "0 sec."

Load INF

Displays the path for the folder from which the .inf file will be loaded.

Save INF

Displays the path for the folder where the .inf file will be saved.

Acquire Image

Specifies whether image acquisition has been enabled.

Update Image

Specifies whether updating of image display has been enabled.

Display Window

Specifies whether the image will be displayed.

Save Image

Specifies whether image saving has been enabled.

Wavelength

Selects the wavelength image for which the wavelength configuration information is to be displayed.

Event Marks

Displays the event marks in the Events List.

OK

Loads the selected protocol file.

Cancel

Cancels the command.

Save Protocol File (File Menu)

Saves the on-screen position of each dialog box and image window, and the current selection of configuration options (illumination, acquisition, etc.).

Use this command to save a protocol file. A protocol file stores each dialog box's selected options and position on-screen, as well as all acquisition and display settings. Saving a protocol file for each type of experiment (ratio, single wavelength, and Delta F/F) will eliminate the need to reconfigure such options as data logging or acquisition, and you can configure MetaFluor to display its settings each time you complete an experiment.

When you save a protocol file, the Save Protocol dialog box will appear. MetaFluor will add the extension ".fsf" to your file name.

Protocol files can be loaded using the Load Protocol File command. If you want to be prompted to load a protocol file whenever you open an experiment or start a new one, you can configure an option in the General Preferences dialog box to do so (Preferences command, File menu).

The settings for the currently active protocol file can be viewed at any time using the Get Info command. Both the Save Protocol File and Get Info commands can save the protocol settings to a text file, copy them to the Clipboard, or send them to a printer.

See Also:

Preferences

Load Protocol File

Get Info

Saving a Protocol File

To save a protocol file, use the following procedure:

Step	Action
1	From the File menu, choose Save Protocol File. The Save Protocol dialog box will appear.
2	If you wish, type an annotation in the *Description* text box.
3	If you want to print or export the information in the Save Protocol dialog box, choose *Export*. The Export dialog box will appear. Choose
	Save Info to a Text File if you want to store the information as a text file. The export Info to File dialog box will appear. Type a name for the file in the *File Name* text box, use the *Save In* list or Up One Level button to select the drive and folder if necessary, and choose *Save.*
	Copy Info to Clipboard if you want to copy the information to the Clipboard for pasting into another Windows-based program.
	Print Info if you want to send the information to a printer.
4	Choose *Save.* The Save Protocol dialog box will appear.
5	Select the desired directory. If necessary, use the *Save In* list or Up One Level button to change to the correct location.
6	Type the desired name in the *File Name* text box. For example, a protocol file for a Delta F/F experiment could be named "DeltaF." MetaFluor will add the file extension ".fsf" to your file name.
7	Choose *Save.* The Select Directory dialog box will close and the Save Protocol dialog box will reappear.
8	When you have finished, click the Close button in the upper right corner of the Save Protocol dialog box to close it.

Saving a Protocol File and Closing an Experiment

The best time to save a protocol file is right after you have completed an experiment since all of the appropriate dialog box options are already selected.

To save a protocol file, use the following procedure:

Step	Action
1	From the File menu, choose Close Experiment. A dialog box will appear.
2	Choose *Yes* to close the experiment and save the protocol. The Save Protocol dialog box will appear.
3	If you wish, type an annotation in the *Description* text box.
4	If you want to print or export the information in the Save Protocol dialog box, choose *Export*. The Export dialog box will appear. Choose *Save Info to a Text File* if you want to store the information as a text file. The export Info to File dialog box will appear. Type a name for the file in the *File Name* text box, use the *Save In* list or Up One Level button to select the drive and folder if necessary, and choose *Save.* *Copy Info to Clipboard* if you want to copy the information to the Clipboard for pasting into another Windows-based program. *Print Info* if you want to send the information to a printer.
5	Choose *Save.* The Save Protocol dialog box will appear.
6	Select the desired directory. If necessary, use the *Save In* list or Up One Level button to change to the correct location.
7	Type the desired name in the *File Name* text box. For example, a protocol file for a Delta F/F experiment could be named "DeltaF." MetaFluor will add the file extension ".fsf" to your file name.
8	Choose *Save.* The Select Directory dialog box will close and the Save Protocol dialog box will reappear.
9	When you have finished, click the Close button in the upper right corner of the Save Protocol dialog box to close it.

Save Protocol - Dialog Box Options

Description

Use this text box to type any comments or annotation that you want to attach to the protocol file.

Timelapse Interval

Displays the time interval between image acquisitions. If images were not acquired in timelapse fashion, this will read "0 sec."

Load INF

Displays the path for the folder from which the .inf file will be loaded.

Save INF

Displays the path for the folder where the .inf file will be saved.

Acquire Image

Specifies whether image acquisition has been enabled.

Update Image

Specifies whether updating of image display has been enabled.

Display Window

Specifies whether the image will be displayed.

Save Image

Specifies whether image saving has been enabled.

Exposure Time

Displays the exposure time for the selected wavelength image (digital acquisition).

Camera Gain

Displays the gain setting if your digital camera supports this feature.

Illum. Device

Displays the name of the installed Illumination MetaDevice used for the selected wavelength image.

Wavelength

Displays the wavelength of illumination for the selected wavelength image.

Intensity

Displays the intensity of illumination for the selected wavelength image.

Use Shutter

Displays the shutter usage status *(Yes* or *No)* for the selected wavelength image.

Wavelength

Selects the wavelength image for which the configuration, acquisition, and display information is displayed. Most of these settings were specified in the Configure Acquisition dialog box (Configure menu).

Event Marks

Displays the event marks in the current *Events List.*

Save

Opens the Save Protocol dialog box, from which you can select a file name, folder, and drive for the protocol file. The file will be saved when you choose *Save* and return to the Save Protocol dialog box.

Export

Opens the Export dialog box, from which you can save the information to a text file, copy it to the Clipboard, or send it to a printer.

Cancel

Cancels the command.

Archive Experiment (File Menu)

Compresses a selected set of files pertaining to an experiment using PKZIP compression software.

Use this command to create a PKZIP file that compresses an experiment's files. The archive file can be stored anywhere, such as on an optical memory disk recorder (OMDR) drive. You can also use this command to append to an existing archive file when you need to add new files or replace existing files in it. You must type an *Archive Description* in the dialog box before archiving the experiment.

When an .inf file is selected as a source file, MetaFluor will automatically select the images associated with that experiment file so that you do not need to mark each image file separately when marking files for the compressed file.

An archived experiment can be extracted using the Extract Archived Files command.

See Also:

Extract Archived Files

Archiving an Experiment

To archive an experiment, use the following procedure:

Step	Action
1	From the File menu, choose Archive Experiment. The Archive Experiment dialog box will appear.
2	Choose *Source File Directory.* The Browse for Folder dialog box will appear. Select the folder which contains the files you want to archive and choose *OK* to return to the Archive Experiment dialog box. The files in your selected folder will appear in the *Source Files* table.
3	To mark files for the archive, select the desired file name in *Source Files* table so that it is highlighted. Then choose *Toggle Check On/Off.* A check mark will appear before the file's name. Repeat for each file you want to add. **OR** Double-click a file's name to select or clear the check mark.
4	To choose the location and file name for the compressed file, choose *Archive Location.* The Archive Location dialog box will appear. Use the *Save In* list to select the desired location. Then type the file name for the compressed file in the *File Name* text box. Choose *Save.*
5	When you have marked the desired files for the archive, type a description for the archive in the *Archive Description* text box.
6	If the archive file already exists, the Archive Exists dialog box will appear. Choose *Freshen* to add files to an existing archive or *Replace* to replace the entire archive with new files.
7	A confirmation message will appear, informing you of the number of files and the size of the archive file. Choose *Archive* to compress the selected files into a single archive (*.zip) file.

Archive Experiment - Dialog Box Options

Source File Directory

Opens the Browse for Folder dialog box, from which you can selects the folder containing the source files that are to be archived. The files in your selected folder will appear in the *Source Files* table.

Archive Location

Specifies the name of the archive file and the location for storage of the file. Use the *Save In* list to specify the location. You can create a new subfolder by clicking the Create New Folder button, if necessary. Then type the name of the archive file in the *File Name* text box. Choose *Save* when you have finished. After you have specified an archive file, its name and location will be displayed next to the *Archive Location* command button.

Source Files

Lists the files in the source file directory. Only those marked with a check mark will be archived when you choose *Archive.* Use the commands below the *Source Files* list to enable/disable the check marks. You can also double-click the desired file names to enable/disable the check marks.

Source File Description

Displays a description of the selected file. If an .inf file is selected, the number of image files associated with the experiment will also be displayed in the description.

Archive Description

Lists your choice of a description for the archive. You must type a description in this text box before archiving an experiment.

Toggle Check On/Off

Enables or disables the check mark for the selected source file name.

Check All On

Enables the check marks for all files in the directory.

Check All Off

Clears the check marks from all files in the directory.

Archive

Compresses the marked files in an archive file. If an existing archive file is selected, this command can be used to freshen (add to) the archive or completely replace the contents of the archive.

Cancel

Cancels the command.

Extract Archived Files (File Menu)

Extracts files from an archive file using PKUNZIP decompression software.

Use this command to extract files that were compressed using the Archive Experiment command. This command displays the names of all files in a compressed file. The Extract Archived Files command displays the amount of space that the files will occupy after they are extracted, as well as the space currently available on the selected destination drive.

See Also:

Archive Experiment

Extracting Archived Files

To extract files which have been archived, use the following procedure:

Step	Action
1	From the File menu, choose Extract Archived Files. The Extract Archived Files dialog box will appear.
2	To select the location of the archive file you want to extract, choose *Archive Directory.* The Browse for Folder dialog box will appear.
	Select the folder which contains archived file that you want to decompress. Then choose *OK* to return to the Extract Archived Files dialog box. Any archive files residing in the selected folder will appear in the *Archives* table.
3	To choose the location and file name for the extracted files, choose *Extracted Files Directory.* A second version of the Browse for Folder dialog box will appear.
	Select the folder where you want the extracted files to reside. If necessary, choose *New* to create a new folder, and type a name for the folder in the text box which appears next to the new folder's icon. Then choose *OK* to return to the Extract Archived Files dialog box.
4	Select the archive file that you want to extract from *Archives* table.
	The name of the files in the archive file will be displayed in the *Archive Contents* list.
5	Choose *Extract.*
	If a file to be extracted has the same name as an existing file in the folder, you will be asked if you want to rename the file or overwrite it. When the file(s) are extracted, a confirmation message will be displayed. Choose *OK.*

Extract Archived Files - Dialog Box Options

Archive Directory

Opens the Browse for Folder dialog box, from which you can select the folder containing the archive file to be unzipped. Any archive files residing in the selected folder will appear in the *Archives* table.

Extracted Files Directory

Opens the Browse for Folder dialog box, from which you can specify a location for the extracted files. If necessary, choose *New* to create a new folder, and type a name for the folder in the text box which appears next to the new folder's icon. When you have specified a folder for the extracted files, its name will be displayed next to the *Extracted Files Directory* command button.

Archives

Lists the names of the archive files in the selected archive directory. The highlighted folder will be the one that is extracted.

Archive Contents

Displays a list of the names of the files contained in the archive file. For compressed experiment files, this includes both the .inf files and the image files.

Archive Description

Displays the archive's description.

Extracted Files from This Archive Will Occupy

Displays the amount of space needed for the files in the archive when they are extracted.

Disk Space Available on Selected Drive

Displays the amount of disk space currently available on the selected drive.

Extract

Extracts the archive file selected in the *Archives* list.

Close

Closes the dialog box.

Print Image (File Menu)

Prints the active image to the selected printer.

Use the Print Image command to print images if a printer is available. You can select the exact size and position of the image on the printed page. You can also choose whether or not to print the name of the image (which you can change) at the top of the page, and whether or not to include the overlay information (thresholding overlays, object overlays, graphics, and so on).

The image will be printed as displayed. Thus, if the Scale 16-Bit Images command has been applied to a 16-bit image prior to printing, the scaled "8-bit" view of the image will be printed.

Note: All images that are printed with their object overlays will be rendered using 24-bit pixel encoding (even 8-bit images and scaled 16-bit images). Because this requires three times as much printer memory as an 8-bit image, and because a temporary buffer is used to render the image, you must make certain that your printer has sufficient memory to print full-page graphics.

See Also:

Scale 16-Bit Images

Printing an Image

To print an image, use the following procedure:

Step	Action
1	From the File menu, choose Print Image. The Microsoft Windows standard Print dialog box will appear.
2	The currently selected printer will be displayed at the top of the dialog box. If you do not want to use this printer, choose *Properties* and select a different printer from the *Name* list in the Print Setup dialog box that appears. Then choose *OK* to return to the Print dialog box.
3	Select *All* from the *Print Range* group.
4	Select the desired number of copies you want to print using *Number of Copies.*
5	If you want to specify an image size and position that differs from the default (full page, centered), choose *Options* and select your alternative settings from the **Print Options dialog box.** Then choose *OK.*
6	If you want to print the image overlays, select the *Print Image Overlay Information* check box so that a check mark appears in it.
7	Choose *OK* to close the Print Options dialog box and return to the Print dialog box.
8	Choose *OK* to carry out the print command.
9	MetaFluor will display a status box while printing the image.
	You can choose *Cancel* from the status box to stop the printing.
	Note: If Windows has started downloading the print job to the printer when you choose *Cancel,* you may need to wait for the printer to finish, or you may need to reset the printer manually.

Selecting Printing Options

To change the Print Option settings for the image to be printed, use the following procedure:

Step	Action
1	From the Print dialog box, choose *Options.* The Print Options dialog box will appear.
2	If you want to include the image title with the printed image, select *Yes* from the *Print Title* radio button group. If necessary, you can type a different title in the accompanying text box. **OR** If you want to omit the image title, select *No.*
3	Use the *Left* and *Top* text boxes in the *Position* group to specify the distance, in inches, between the upper left corner of the paper and the left and top edges, respectively, of the printed image. Alternatively, you can drag the box-in-box, displayed at the left of the Print Options dialog box, to manipulate the placement of the image on the printed page. **OR** If you want to center the image on the printed page, choose *Center.*
4	Use the *Width* and *Height* text boxes in the *Size* group to specify the horizontal and vertical size, in inches, of the image on the printed page. **OR** If you want the image to be as large as the paper size (and printer) permits, choose *Fill Page.*
5	If there are any regions of interest defined on the image, select a setting for the *Region Outline Color.* You can select either a *White* or *Black* outline, or, if you do not want to draw the regions on the printed image, select *None.*
6	If you want to print the image overlays (segmentation and thresholding overlays, object measurement overlays, etc.), select *Print Image Overlay Information.*
7	If the lines and edges of objects in your image are "jaggy" (i.e., appear "boxy") and you want to use an algorithm to smooth the edges, select the *Interpolate Image to Smooth Pixel Edges* check box. **Note:** This algorithm works by increasing the number of pixels and interpolating where the newer, intervening pixels should be. Image overlays can not be expanded in this manner. Consequently, the *Interpolate Image to Smooth Pixel Edges* option and the *Print*

Image Overlay Information option are mutually exclusive.

8   Choose *OK* to accept the Print Options settings and return to the Print dialog box.

Print - Dialog Box Options

Printer

Lists the current printer.

Print Range

Selects the print range to be printed.

Number of Copies

Selects the number of copies to print.

Collate Copies

When more than one copy of a multiple-page selection is being printed, selecting this check box will direct the printer to print all pages in the first copy of the selection before starting any of the pages for the next copy. When this box is cleared, all copies of a page in the selection will be printed before starting on the next page in the selection.

Properties

If you do not want to use the printer listed in the *Printer* status line, choosing this command button will open the Print Setup dialog box. You can select a different printer from the *Name* drop-down list box .

Options

Opens the Print Options dialog box.

Cancel

Cancels the command.

OK

Starts the printing process and displays a printing status dialog box while the image is printing.

Print Options - Dialog Box Options

Print Title

Specifies whether the image title is to be printed with the image. Select *Yes* to print the title, which you can change by typing a new name in the accompanying text box. Select *No* to omit the title.

Left

Specifies the distance, in inches, from the edge of the page to the left edge of the printed image.

Top

Specifies the distance, in inches, from the top of the page to the top edge of the printed image.

Center

Prints the image directly in the center of the printed page. This is the default setting for image position.

Width

Specifies the horizontal size, in inches, of the printed image.

Height

Specifies the vertical size, in inches, of the printed image.

Fill Page

Prints the image as large as the paper size will permit. This is the default setting for image size.

Region Outline Color

Selects whether the outlines of regions of interest are to be left off of the printed image *(None),* to be drawn in *White,* or drawn in *Black.* The default setting is *None.*

Print Image Overlay Information

When selected, this option allows you to print all image overlays (except region outlines, which can nevertheless be printed using *Region Outline Color)* that are on the image when the image is printed.

Interpolate Image to Smooth Pixel Edges

Applies an algorithm to smooth the lines and the edges of objects in the image. **Note:** This algorithm works by increasing the number of pixels and interpolating where the newer, intervening pixels should be. Image overlays can not be expanded in this manner. Consequently, the *Interpolate Image to Smooth Pixel Edges* option and the *Print Image Overlay Information* option are mutually exclusive.

OK

Accepts the Print Options settings.

Cancel

Cancels any changes to the dialog box settings.

Select Video/Camera for Acquisition (File Menu)

Selects an active video board or camera and video channel from a list of the video devices that have been installed with the Video Driver Manager.

Use this command to change the current video device from the default selection without needing to quit MetaFluor and use the Video Driver Manager. This command's dialog box will display only those video devices that are currently installed. If you want to use a video device that is not currently installed, you must quit MetaFluor and use the Video Driver Manager to install it.

This command temporarily changes the current video device for the current work session; it does not change the default video device selection in the Video Driver Manager.

You can use this command only before opening a new or stored experiment. After you open a stored experiment or start a new experiment, this command will become unavailable.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Video Driver Configuration

Preferences (File Menu)

Changes the default settings for one or more states or actions that occur in the MetaFluor environment to your preferred choice among the available settings.

Use the Preferences command to change settings for options that fall in one of the following categories:

- General,
- Digital Camera,
- Video Camera,
- Scale Bar,
- Calibration,
- Playback,
- Data Logging, and
- Dual View.

In addition, you can use the Initialize to Defaults command in the File menu to return settings to their original state. This command will affect such settings as the automatic appearance of the Status and Notebook windows and the graph configuration settings, but will not affect video or device drivers or image window placement. The Initialize to Defaults command is available only when there is no experiment open or active.

Changes made to the Preferences settings will affect the remainder of the current work session and all subsequent sessions unless you change the settings later.

Note: The Video Camera, Digital Camera, and Focusing Preferences are unavailable in the MetaFluor Offline system.

Selecting Preferences

To select preferences, use the following procedure:

Step	Action
1	From the File menu, choose Preferences. The Preferences dialog box will appear.
2	Select the tab for the type of preferences you wish to configure: General, Digital Camera, Video Camera, Scale Bar, Calibration, Playback, Data Logging, or Focusing.
3	Select the options you want to use. (Refer to the description for each option next to the appropriate check box in each of the Preferences dialog box pages.)
4	Choose *OK* to accept the selected preferences.

General Preferences - Dialog Box Options

Prompt to Select a Protocol File

Opens the Load Protocol dialog box automatically when you open an existing experiment or start a new experiment.

Open the Status Window

Opens the Status window automatically when you open an experiment or start a new experiment.

Open the Notebook Window

Opens the Notebook window automatically when you open an experiment or start a new experiment.

Open the Experiment Control Panel

Opens the Experiment Control panel automatically when you open an experiment or start a new experiment.

Backup the INF File to *.BAK

When this check box is selected, the .inf file will be copied to a backup (*.bak) file at the specified interval of acquisition cycles.

Make Backup Every… Cycles

Specifies the acquisition cycle interval between .inf file backups.

Save Using LZW Compressed TIFF Files

Uses Lempel Ziff compression to store your images. This is a lossless compression (i.e., with no loss of image data) that can save storage space, particularly with images that have less pixel-to-pixel variability of gray values.

Average All Regions of the Same Color

All regions of the same color will be considered one disjoint region.

Draw Labels Next to Region Outlines

Displays numeric labels next to each region of interest.

Region Label

Opens the Regions Label Position dialog box. You can specify the location of the label *(Top, Bottom, Left,* or *Right),* its position inside the region outline, and its foreground and background colors. You can also choose whether or not to fill the label background.

Use Graph Markers

If this option is selected, clicking on a graph displays a line which appears at that location on all graphs, and moves with the pointer when the mouse button is held down. This option allows you to compare data at a given X-axis position in all graphs.

Do Not Clear Graph When Regions Change

When this option is selected, the graphs will not be cleared even when new regions are created or when regions are edited.

Maximum Number of Points on Graph

Specifies the maximum number of points plotted on the MetaFluor graphs.

Allow Repositioning of Acquisition Region When Focusing

When selected, this option permits repositioning of the acquisition region during a focusing procedure in which the image is displayed in an image window. This ability is optional because it may be undesirable when certain cameras are used because some cameras, such as the Hamamatsu 4880-80, take a long time to download the new region.

Ratio Image Display

Selects the method by which ratio images are calculated and displayed in an **Intensity Modulated Display** (IMD). If you select *Display as 8-Bit Image with LUT,* ratio images will be displayed by scaling the IMD display over a range of 256 possible pixel color/intensity values. If you select *Display as 24-Bit Image* (Default value)*,* you will be able to select an IMD overlay display with the Image Display Controls command (Configure menu), using a grayscale image of your choice (either one of the wavelength images, the average of the intensity values at each pixel in all wavelength images, or the maximum intensity value at each pixel in the wavelength images) to represent intensity. The ratio images will be displayed over a range of ~16.7 million color values, using an IMD display that represents the ratio values by the hue in each image pixel. **Note:** This latter method is mathematically intensive and therefore slower. It is thus best suited for display of images from stored experiments.

OK

Accepts any changes to the general preferences.

Cancel

Cancels the command.

Digital Camera Preferences - Dialog Box Options

Current Camera Temperature

Displays the current temperature for the camera. Use the configuration settings in the Meta Imaging Series Administrator to specify the cooling temperature for the camera.

Camera Settings

Sets the Light Mode and Camera Offset values for Hammamatsu cameras.

Light Mode

Sets the camera's light mode to either High or Low.

Camera Offset

Adjusts the black level reference above the zero level to reduce or eliminate background noise.

Chip Clear Counts

Specifies the "Clear Counts" setting for wiping the camera chip before acquisition. The default setting is *2* wipes.

Method of Focusing the Camera

Selects a location for the images that are displayed during the Experiment Control Panel's focusing procedure--either on the computer's monitor *(Update an Image Window on the Screen)* or on an external video monitor *(Use an External Monitor).*

Maximum Bit Depth

Sets the image bit depth. Enables you to expand the camera image bit depth range to fit within the bit depth range of the acquired image. Set this value to 24-Bit to enable color image acquisition from qualified color video cameras.

Reset Camera

Sends a command to the camera to reinitialize it.

Keep Stream Memory

If this box is checked, the contents of memory are retained after streaming is finished.

OK

Accepts any changes to the digital camera preferences.

Cancel

Cancels the command.

Video Camera Preferences - Dialog Box Options

Settling Time (ms) for Changing Analog Values

Specifies the time to wait before acquisition changing the video analog settings.

Settling Time (ms) for Changing Input Channel

Specifies the time to wait before acquisition after changing the video input channel.

Sum Frames Instead of Averaging Frames

Directs MetaFluor to acquire images by summation into a 16-bit image, rather than by averaging. This option will be useful in low-light applications in which averaging yields images that are too dark to discern contrast differences.

Note: This option only applies when you are using a video-rate camera.

Focus using an external monitor if it is attached

Indicates that image focusing will use an optional external monitor connected to the video camera interface. If your video camera interface provides a connection for an external monitor to use for focusing, and you have a monitor connected, click this box.

OK

Accepts any changes to the video camera preferences.

Cancel

Cancels the command.

Scale Bar Preferences - Dialog Box Options

Scale Bar Drawing

Specifies which images should have scale bars drawn on them. Choose one of the following options:

Off – No scale bar

Scale Bar on Ratio Only – Scale bar only on the ratio image

Scale Bar on All Images – Scale bars on all images

Scale Bar Size

Determines the size of the scale bar displayed in the image viewer and the amount of space that it will use. Choices are *Large, Medium,* and *Small.*

Scale Bar Location

Specifies the location of the scale bar as either the left or right side of the image.

Scale Bar Labels

Stamps a scale bar on the image.

Scale Bar Location

Specifies whether the scale bar is to appear on either the left or right side of the image.

OK

Accepts any changes that you made to the preferences dialog box.

Cancel

Closes the Preferences dialog box and disregards any changes that you made on any preference tab.

Calibration Preferences - Dialog Box Options

Ask Whether Backgrounds Should Be Subtracted

When this option is selected, you will be asked whether background reference images should be subtracted when you load calibration reference images.

Default to Subtracting Backgrounds

Specifies the default for background subtraction when loading calibration reference images to subtract the background reference images.

Equation Calibration in vitro:

Provides a checkbox to specify using a higher ratio for lower concentration solutions.

Invert Equation Calibration

Specifies that a higher ration is needed to accommodate a lower concentration.

Equation Calibration Method

Specifies one of two possible methods to use as the Equation Calibration Method.

Standard

Uses a single set of calibration constants for the experiment. This method is recommended when the calibration images are from standard solutions, or the calibration constants are determined from calibrating experimental images.

Normalized per region calibration

Uses a different set of calibration constants for each Region-of-Interest in the experiment. Use this method when an independent Rmin-Rmax dynamic range is assumed for each region or pixel in the image.

OK

Accepts any changes to the calibration preferences.

Cancel

Cancels the command.

Playback Preferences - Dialog Box Options

First Image Is at Original Time

Determines what time is to be assigned to the first image in the series. If image acquisition was not initiated immediately when the New Experiment command was chosen, the first image will have a non-zero time. Selecting the *First Image Is at Original Time* check box will assign that time to the image. Clearing the check box will assign a time of 0.00. Times for all subsequent images will be assigned in accordance with your selection.

Automatically Open Event List on Experiment Playback

Automatically displays a list of events when an experiment is played back.

Display Dialog When an Event Mark Occurs During Playback

Displays a message dialog box notifying the user that an event has occurred during playback.

Erase Graphs If Clock Reset During Playback

Erases the graphs if the clock is reset during playback mode.

Clicking on Graph Displays Image Nearest That Time

If this option is selected, MetaFluor will display the images that correspond to that time on the graph.

OK

Accepts any changes to the playback preferences.

Cancel

Cancels the command.

Data Logging Preferences - Dialog Box Options

Time

Specifies the units of measure for the logged data *(Milliseconds, Seconds, Minutes,* or *Hours).*

Average Value, Integrated Value, Area, Ratio, and **Calibrated Value**

Specifies the formatting used for logging each of these values. You can use the period (.) and the number sign (#). For example, to use three significant digits in the decimal, type "#.###".

Log Header After Editing Regions

Logs regional coordinates, size, and thresholded areas automatically whenever you edit any regions (move, resize, add, remove) with the Define Regions for Measurement command.

OK

Accepts any changes to the data logging preferences.

Cancel

Cancels the command.

Dual View Preferences - Dialog Box Options

A Dual View Emission Splitter is installed.

Check this box to enable the *Configure Dual View* command on the Configure menu.

Orientation

Choose Vertical or Horizontal to specify the orientation of the camera to the emission splitter.

> **OK**
>
> Accepts any changes to the focusing preferences.
>
> **Cancel**
>
> Cancels the command.

Initialize to Defaults (File Menu)

Returns the MetaFluor system settings to their default states.

Use this command to return settings to their original "factory-configured" state. This command will affect such settings as the automatic appearance of the Status and Notebook windows and the graph configuration settings, but will not affect video or device drivers or image window placement. The Initialize to Defaults command is available only when there is no experiment open or active.

See Also:

Load Protocol File

Preferences

Initializing MetaFluor Settings to Their Default State

To reset the MetaFluor system settings back to their "factory" default settings, use the following procedure:

Step	Action
1	From the File menu, choose Initialize to Defaults. A message box will appear, asking you to verify that you want to reset the settings back to their defaults.
2	Choose *Yes.* The settings will be reset.

Configure Paths (File Menu)

Configures the default directory paths for the location of each type of file that is opened or saved in MetaFluor.

Use this command to specify the default paths for file types that are frequently saved in the same directory location. For example, if you always save journal toolbars to a directory called \MM\Apps\MMFluor\Toolbars, you can use this command to configure the default path for Journal Toolbars so that whenever you create or load a journal toolbar, the \MM\Apps\MMFluor\Toolbars directory will appear in the dialog box as the default directory.

See Also:

Save Protocol File

Configuring Paths

To configure your default paths, use the following procedure:

Step	Action
1	From the File menu, choose Configure Paths. The Configure Paths dialog box will appear.
2	Pick the entry in the *MetaFluor Path* column that you want to change. **OR** If you want to use the same path for several file types, select the corresponding entry check boxes in the *MetaFluor Path* column.
3	Choose the *Set* button for the selected entry. **OR** If you selected several check boxes in Step 2, choose the *Set* button for any of the entries for which you checked a *MetaFluor Path* check box. The Browse for Folder dialog box will appear.
4	Select the appropriate folder for the type of files you selected in the *MetaFluor Path* list. Then choose *OK* to return to the Configure Paths dialog box.
5	By default, the path that has been stored in a protocol file will supersede the path you set here. If you want the path you are configuring here to be used, rather than the paths indicated by protocol files, click the *In Protocol* button to toggle it to read *"Globally."* If necessary, another click will toggle the button back to the *"In Protocol"* state.
6	Repeat Steps 2 - 5 for each entry that you want to configure. **OR** If you selected several check boxes in Step 2, choose *Set Checked Paths to Current Directory.*
7	When you have finished, choose *Close.*

Configure Paths - Dialog Box Options

MetaFluor Path

Selects a file type used by MetaFluor for which you want to modify the default path. The check boxes allow you to select more than one file type, which you can then set to the same directory path by choosing the *Set Checked Paths to Current Directory* button.

Directory Path

Indicates the current directory path setting for the entry.

Set

Opens the Browse for Folder dialog box, from which you can select the desired drive and folder for the file type.

Path Saved

Selects how the path configuration will be saved. By default, paths that have been saved in protocol files will supersede the path settings configured in the Configure Paths dialog box *(In Protocol).* Clicking this button toggles it to the *Globally* state, which will force the use of the path configured here to be used, rather than the paths stored in the protocol file. Another click of this button toggles it back to *In Protocol.*

Set Checked Paths to Current Directory

Configures the current directory path for all file types whose check boxes have been selected in the *MetaFluor Path* column. At least one check box must be selected for this button to become available.

Clear Check Marks

Clears all check boxes in the *MetaFluor Path* column. At least one check box must be selected for this button to become available.

Close

Closes the dialog box.

Exit (File Menu)

Closes any experiments that are currently open or running and quits the MetaFluor imaging program.

Use the Exit command to end the current work session and to quit the MetaFluor program before working with another Windows-based program or exiting Windows. In general, it is best to close the current experiment before exiting. MetaFluor will save the default protocol file when exiting, but you must save custom protocol files while closing the current experiment.

TIP: To exit quickly, click the Close button in upper right corner of the MetaFluor title bar.

See Also:

Close Experiment

Exiting MetaFluor

To exit MetaFluor, use the following procedure:

Step	Action
1	From the File menu, choose Exit.
2	If an experiment is still open, MetaFluor will direct you to close the experiment. Select:
	Yes to close the experiment and save a the current custom protocol,
	No to close the experiment without saving the current protocol, or
	Cancel to cancel the Close Experiment and Exit commands.
3	MetaFluor will then ask you to confirm the Exit command. Choose *Yes* to quit the MetaFluor program.

Configure Menu

Configure Acquisition (Configure Menu)

Assigns the wavelength and intensity of the illumination to be used when acquiring each wavelength image. Configures the camera parameters and acquisition region for each image. You can also use the Configure Acquisition command to assign a name to each of the wavelength images.

When configuring a wavelength, you need to select an Illumination MetaDevice, the desired illumination wavelength and/or intensity values, the Z-motor device (if applicable), and the shutter that you will use (if applicable). If you are using a "video rate" camera, you can also select the appropriate video channel for that wavelength.

For a typical fura-2 ratioing experiment using a dual filter wheel, this command allows you to select an excitation wavelength filter and a neutral density filter for each wavelength. Depending on the hardware available and the type of experiment you are performing, you could also set the laser line and power for illumination, the wavelengths of a monochromator, or the position of an emission filter wheel.

This command also enables you to assign a different Z Position to each wavelength to enable you to compensate for refractive differences in image focus caused by wavelength transmission property differences.

Notes:

❑ This command is unavailable when you are playing back images stored on disk.
❑ This command is unavailable in the MetaFluor Offline system.

See Also:

Configure Illumination

Configuring Acquisition for Digital Cameras

To configure acquisition parameters for digital cameras, use the following procedure:

Step	Action
1	From the Configure menu, choose Configure Acquisition. The Configure Acquisition dialog box will appear.
2	Select *Wavelength 1* from the *Wavelength to Configure* list.
3	If you want to rename the image window, select *Custom Defined* from the *Wavelength Name* option button group and type the new name in the accompanying text box.
4	Select the desired MetaDevice from the *Illumination MetaDevice* list.
5	If you need to specify a Z position to be associated with this wavelength, click *Set Z position for this wavelength to* and type the specific Z position in the box. For information about setting the Z position, see the help for the **Z Position Control** dialog box.
6	If you want to use an acquisition subregion, use the *Left, Top, Width,* and *Height* spin boxes in the *Camera Binning and Sub-Region* group to select the region's starting (upper left) X and Y coordinate, width, and height, respectively. Alternatively, you can choose **Select Region.** This will display a restricted-mode image window that contains a region with draggable borders. The acquisition region will be defined proprotionally such that, if you change the size of the overall image (for example by changing binning), the region will remain the same relative size and in the same relative location on the image. After you configure the region, choose *OK* from the Select Region dialog box that also appeared.
	OR If you want to use the entire camera chip for acquisition, choose *Use Entire Image* from the Configure Acquisition dialog box.
7	If your camera supports binning, you can select values for pixel binning with the *Binning* spin box.
8	Using *Exposure Time,* select the length of time for each acquisition. **Note:** If you want to use different exposure times for each wavelength image, select *Allow Items to Differ for Each Wavelength.*
9	If available for your camera, you can select settings for *Gain, Bit Depth,* and *Transfer*

Speed.

> **Note:** If you want to use different settings for each wavelength image, select *Allow Items to Differ for Each Wavelength.*

10 Optionally set an appropriate *Intensifer Gain* level if your camera supports this feature.

11 Select the next wavelength from the *Wavelength to Configure* list. Repeat Steps 3 - 11, as needed, for each wavelength

12 Choose *Close* when you have finished.

Configure Acquisition - Dialog Box Options (Digital Camera)

Wavelength to Configure

Specifies the wavelength image that you are configuring for acquisition. Each wavelength image can have separate acquisition settings.

Wavelength Name

Enables you to assign a custom name to each wavelength that you will use.

Use Selected Wavelength as Name

Accepts the *"Wavelength X"* entry as the name to use for the wavelength image name. This name will also be used by such commands as Configure Experiment and Image Display Controls.

Custom Defined

Specifies an alternate name for the corresponding wavelength image. This name will also be used by such commands as Configure Experiment and Image Display Controls.

Illumination Device

Selects the Illumination Device to use for the specified wavelength image. The Illumination device is defined in the Configure Illumination dialog box. Use the Configure Illumination dialog box to specify the wavelength, intensity, and shutter settings for each wavelength.

Z Position (Set Z Postion for this Wavelength to)

Specifies the Z position setting to be associated with the current wavelength. Use this setting if spectral characteristics of the optics dictate the use of a different focal plane for the selected wavelength, and/or when using automated filter cube changers when non-par-focal.

Camera Binning and Sub-Region

Use the following settings to specifiy the area of the image to which you want to assign binning and the binning value.

Left

Specifies the leftmost point of the region.

Top

Specifies the topmost point of the region.

Width

Specifies the width of the region.

Height

Specifies the height of the region.

Binning

Specifies the number of pixels in the horizontal and vertical direction for binning. For example, if you select *2,* a 2 x 2 pixel binning will be used during image acquisition. This will speed up acquisition by a factor of four, and will reduce the amount of memory used by 75%, but image resolution also will be reduced by a similar amount.

Note: If you have already acquired reference (background subtraction or shading correction) images and then change the binning, MetaFluor will check for mismatches between the binning setting of the reference and data images. If a mismatch is detected, any mismatched reference images will be discarded, and a message box will be displayed that informs you of this.

Image Size

This status line will display the size of the acquired images, taking the acquisition region's width and height and the binning into account.

Select Region

Allows you to **specify the region** used for acquisition. The acquisition region will be defined proprotionally such that, if you change the size of the overall image (for example by changing binning), the region will remain the same relative size and in the same relative location on the image.

Use Entire Image

Defines the acquisition region as the entire image.

> **Note:** If you have already acquired reference (background subtraction or shading correction) images and then change the size of the acquisition region, MetaFluor will detect mismatches between the sizes of the reference and data images. Any mismatched reference images will be discarded, and a message box will be displayed that informs you of this.

Digital Camera Acquisition Parameters

Use the settings in this box to specify the settings that will be used by your digital camera.

Exposure Time

Specifies the length of time for each acquisition. When *Allow Items to Differ for Each Wavelength* is selected, you can set separate exposure times for each wavelength.

Gain

If your camera supports this feature, this option sets the gain. When *Allow Items to Differ for Each Wavelength* is selected, you can set separate settings for each wavelength image.

Bit Depth

If your camera supports this feature, this option sets the bit-depth. When *Allow Items to Differ for Each Wavelength* is selected, you can set separate settings for each wavelength image.

Transfer Speed

If your camera supports this feature, this option specifies the camera's speed. When *Allow Items to Differ for Each Wavelength* is selected, you can set separate settings for each wavelength image.

Camera Shutter

Selects a state for the shutter: *Open for Expose, Always Open,* or *Always Closed.* When *Allow Items to Differ for Each Wavelength* is selected, you can set separate settings for each wavelength image.

Intensifier Gain

Optionally set this gain value for digital cameras that contain and support this feature such as the Roper Scientific Photometrics Cascade camera. You can set this as a different value for each separate wavelength.

Allow items to differ for each wavelength

When this option is selected, you can configure different settings for the acquisition region, *Exposure Time, Gain, Bit Depth, Transfer Speed,* and *Camera Shutter* for each wavelength image.

Close

Closes the dialog box.

Configuring Acquisition for Video Cameras

To configure acquisition parameters for video cameras, use the following procedure:

Step	Action
1	From the Configure menu, choose Configure Acquisition. The Configure Acquisition dialog box will appear.
2	Select *Wavelength 1* from the *Wavelength to Configure* list.
3	If you want to rename the image window, select *Custom Defined* from the *Wavelength Name* option button group and type the new name in the accompanying text box.
4	Select the desired MetaDevice from the *Illumination MetaDevice* drop-down list.
5	If you need to specify a Z position to be associated with this wavelength, click *Set Z position for this wavelength to* and type the specific Z position in the box. For information about setting the Z position, see the help for the **Z Position Control** dialog box.
6	If you want to use an acquisition subregion, use the *Left, Top, Width,* and *Height* spin boxes in the *Camera Binning and Sub-Region* group to select the region's starting (upper left) X and Y coordinate, width, and height, respectively. Alternatively, you can choose **Select Region.** This will display a restricted-mode image window that contains a region with draggable borders. After you configure the region, choose *OK* from the Select Region dialog box that also appeared. **OR** If you want to use the entire camera chip for acquisition, choose *Use Entire Image* from the Configure Acquisition dialog box.
7	If your camera supports frame averaging, use the *Frames to Average* spin box to select the number of frames to be averaged for each image. **OR** If you selected the *Sum Frames Instead of Averaging Frames* check box in the Video Camera Preferences dialog box before starting the experiment, use the *Frames to Sum* spin box to select the number of frames to be summed for each image. **OR** If you are using an integrating camera, use the *Frames to Integrate* spin box to select the number of frames to be integrated on-chip for each image.

8 From the *Video Channel* drop-down list, select the video channel to which your camera is connected.

9 Select the next wavelength from the *Wavelength to Configure* list. Repeat Steps 3 - 9 for each wavelength, as needed.

10 Choose *Close* when you have finished.

Configure Acquisition - Dialog Box Options (Video Camera)

Wavelength to Configure

Specifies the wavelength image that you are configuring for acquisition. Each wavelength image can have separate acquisition settings.

Wavelength Name

Enables you to assign a custom name to each wavelength that you will use.

Use Selected Wavelength as Name

> Accepts the *"Wavelength X"* entry as the name to use for the wavelength image name. This name will also be used by such commands as Configure Experiment and Image Display Controls.

Custom Defined

> Specifies an alternate name for the corresponding wavelength image. This name will also be used by such commands as Configure Experiment and Image Display Controls.

Illumination Device

Selects the Illumination Device to use for the specified wavelength image. The Illumination device is defined in the Configure Illumination dialog box. Use the Configure Illumination dialog box to specify the wavelength, intensity, and shutter settings for each wavelength.

Z Position (Set Z Position for this Wavelength to)

Specifies the Z position setting to be associated with the current wavelength. Use this setting if spectral characteristics of the optics dictate the use of a different focal plane for the selected wavelength, and/or when using automated filter cube changers when non-par-focal.

Camera Acquisition Region

Specifies the location and dimensions of the acquisition region.

Left

> Specifies the leftmost point of the acquisition region.

Top

> Specifies the topmost point of the acquisition region.

Width

> Specifies the width of the acquisition region.

Height

> Specifies the height of the acquisition region.

Select Region

> Allows you to **specify the region** used for acquisition.

Use Entire Image

> Defines the acquisition region as the entire image. **Note:** If you have already acquired reference (background subtraction or shading correction) images and then change the size of the acquisition region, MetaFluor will detect mismatches between the sizes of the reference and data images. Any mismatched reference images will be discarded, and a message box will be displayed that informs you of this.

Frames to Average

Specifies the number of frames to be averaged for each image during acquisition.

Frames to Sum

Specifies the number of frames to be summed together (in software) for each image during acquisition.

Frames to Integrate

Selects the number of video frames to be integrated on-chip. This option will appear dimmed unless you have an integrating camera and configured the appropriate settings with the Video Driver Manager program.

Video Channel

Specifies the video channel to use for the acquisition.

Allow Items to Differ for Each Wavelength

When this option is selected, you can configure different acquisition settings for each wavelength image.

Close

Closes the dialog box.

Selecting a Region for Acquisition

To select the region for acquisition, use the following procedure:

Step	Action
1	After you have chosen *Select Region* from the Configure Acquisition dialog box, MetaFluor will close all windows and dialog boxes temporarily. It will open the Select Region dialog box and an image window with a region drawn on it.
2	Choose *Update Image* if you want to update the image window to display the most current image which will assist you in defining the region accurately.
3	If you have created regions of interest and want to use the distribution of the regions to define the acquisition region, select the *Show Measurement Regions* check box. Then choose *Area of Chip Enclosing All Measurement Regions*. The region outlines will be displayed in red, and a yellow region will be displayed which encloses all defined regions. **OR** If you want to acquire the entire image area, choose *Full Chip* from the Select Region dialog box.
4	Adjust the acquisition region as desired, by dragging the region outline in the image window to define the desired size and position for acquisition. (The region's position and size will be displayed in the Select Region dialog box's *Pos* and *Size* status fields, respectively.)
5	After you have finished editing or defining regions, choose *OK.* MetaFluor will restore the image windows and dialog boxes that were previously open.

Select Region - Dialog Box Options

Update Image

Acquires an image for display in the acquisition region configuration image window.

Full Chip

Creates an acquisition region that is the size of the entire chip and shrinks or enlarges the acquisition region by a factor of two.

Note: If you have already acquired reference (background subtraction or shading correction) images and then change the size of the acquisition region, MetaFluor will detect mismatches between the sizes of the reference and data images. Any mismatched reference images will be discarded, and a message box will be displayed that informs you of this.

Center

Centers the acquisition region on the chip.

Area of Chip Enclosing All Measurement Regions

Creates an acquisition region that encloses all currently defined regions of interest. The outline of the acquisition region can be adjusted by dragging its borders.

Show Measurement Regions

Displays any currently defined regions of interest in the acquisition region configuration image window.

Pos

Displays the X and Y coordinate of the upper left corner of the acquisition region.

Size

Displays the X and Y dimensions of the acquisition region

OK

Accepts the current configuration of the acquisition region and closes the dialog box.

Cancel

Rejects any changes made to the configuration of the acquisition region and closes the dialog box.

Configure Experiment (Configure Menu)

> **Enables/disables image acquisition and display for each image and configures the frequency of acquisition and display. Specifies the data to be logged for each defined region of interest.**

Use this command to enable or disable acquisition or display of each image. You can configure each image window separately by selecting or clearing the individual check boxes. Alternatively, you can simultaneously switch off acquisition, display, saving, and data logging for an image by choosing the *On/Off* button for that image. Although you may not use this command often, the ability to switch acquisition and display on or off gives you the flexibility to conduct Delta F/F experiments or those that require short acquisition times.

You can specify the frequency of image acquisition and display from the Configure Experiment dialog box. For example, if you set the acquisition frequency for Wavelength 1 to *5,* Wavelength 1 would be acquired only once every five cycles. For the intermediate cycles, MetaFluor would use the last Wavelength 1 image that was still stored in memory.

Note: The *Acquire* check box and the *Acquire Interval* check box are available only in acquisition mode, and will not be displayed in playback mode.

If you are using an external video monitor, you can display the Wavelength 1, Wavelength 2, Wavelength 3, and Ratio images simultaneously during acquisition using a quadrant display (as if they were in separate image windows). For faster acquisition, you can choose to display just one image on the monitor, rather than using the quadrant display.

You can also set different frequencies for saving each type of wavelength or ratio image. For example, you can set the interval for saving Wavelength 1, 2, and 3 images to five acquisition cycles. When you enable image saving in the Experiment Control Panel, MetaFluor will save a set of wavelength images to disk on every fifth acquisition cycle. If you set a saving interval other than *1,* the status text next to *Save Images* and/or *Save Ratios* in the Experiment Control Panel will list the interval. If you want to adjust the saving frequency while running an experiment, just leave the Configure Experiment dialog box open while using the Experiment Control Panel. You can set an interval of *0,* in which case that image will not be saved. For example, if you are running a single-wavelength experiment, you could set the Save Interval to *0* for Wavelengths 2 through 5, and set the Save Interval for Wavelength 1 to *1.* In this configuration, when you save images, you will save only the first wavelength image. This will reduce the amount of disk space you will need to store the experiment.

Note: This command does not initiate image saving; it merely enables it. To save images, you must also use the Open Save Images File or Open Save Ratios command and select the *Save Images* or *Save Ratios* check box in the Experiment Control Panel.

This command also specifies the data to be logged for each region. You can select the data to be logged individually for each image. For wavelength images, you can enable or disable logging of the average intensity, integrated intensity (i.e., the sum of the grayscale values for all pixels), and the area of the thresholded portion of each region of interest. For ratio images, you can enable or disable logging of ratio values. If you have calibrated an image series, you can enable logging of the calibrated data.

Note: This command does not enable data logging. You must still open a measurements file by using the **Open Measurements File** command and enable data logging by using the Experiment Control Panel.

See Also:

Experiment Control Panel:

For Video Camera with External Monitor

For Video Camera with Computer's Monitor

For Digital Camera

For Playback

Open Save Images File

Open Save Ratios File

Open Measurements File

Configuring Acquisition, Display, Saving, and Data Logging for an Experiment

To configure acquisition, display, saving, and data logging for an experiment, use the following procedure:

Step	Action
1	From the Configure menu, choose Configure Experiment. The Configure Experiment dialog box will appear.
2	If you are playing back a stored experiment, skip to Step 5. **OR** If you are acquiring images in a new experiment, continue to Step 3.
3	To enable image acquisition for Wavelength 1, select the *Acquire?* check box in the first row. **AND** Select an acquisition frequency from the *Acquire Interval* spin box in the first row. For example, to specify acquisition on every cycle, select *1.* Likewise, select *5* to specify acquisition of this image once every five cycles.
4	From the *Save Interval* spin box, select a saving interval. For example, to save the image once every five acquisition cycles, select *5.* You can also set an interval of *0,* in which case the image will not be saved.
5	To enable display of the image, select the *Show?* check box. **AND** Select the *Update?* check box to allow the image to be refreshed with updated images. Then select an updating interval from the *Update Interval* spin box.
6	Repeat Steps 4 and 5 (or Steps 3 - 5 if you are acquiring new images) for each wavelength and ratio image, selecting or clearing the pertinent check boxes and selecting the appropriate frequencies of acquisition, saving, and updating. **Note:** To disable all options for a particular wavelength or ratio image, choose the pertinent *On/Off* button. The *Acquire* check box for that image will be cleared and all other options will be disabled and will become unavailable. To reenable use of an image, choose the *On/Off* button again. The options will become available once again.
7	From the *Data Logging* columns, select the

types of region data you want to log.

For wavelength images, you can enable logging of the average intensity value *(Avg),* the integrated intensity *(Sum),* and/or the area of the thresholded portion of the region *(Area).*

For ratio images, you can enable logging of the average *Ratio* value.

8 If you have already performed a calibration, you can enable logging of the calibrated data by choosing the *On/Off* button for the *Calibrated* image to enable the *Calibrated Value* check box, or by directly selecting the *Calibrated Value* check box.

Note: The most recently calibrated image (as performed with the Acquire Calibration Standards command) will be the one measured and analyzed. The calibrated image will be indicated in the accompanying status text for the *Calibrated* image.

9 When you have finished, choose *OK* to close the dialog box.

Note: If you plan to change the acquisition frequency or saving intervals, you may wish to leave this dialog box open.

Configure Experiment - Dialog Box Options

On/Off

Enables or Disables all options for the associated wavelength or ratio image. When turned off, the *Acquire* check box for that image will be cleared and all other options will be disabled and made unavailable. To reenable use of the image, click the *On/Off* button again.

Acquire?

Configures MetaFluor to acquire the associated wavelength image at the interval specified by the *Acquire Interval* spin box. This option appears only in acquisition mode, and will not be displayed in playback mode.

Acquire Interval

Selects an acquisition interval for the associated wavelength image. For example, to specify acquisition on every cycle, select *1.* Likewise, select *5* to specify acquisition of this image once every five cycles. This option appears only in acquisition mode, and will not be displayed in playback mode.

Avg

When data logging has been enabled, this check box prompts MetaFluor to save the average grayscale value for all pixels in each region of interest that has been defined.

Sum

When data logging has been enabled, this check box prompts MetaFluor to save the integrated grayscale value for each region of interest (i.e., the sum of the grayscale values for every pixel in the region).

Area

When data logging has been enabled, this check box prompts MetaFluor to save the area of the thresholded portion of each region of interest that has been defined.

Ratio

When data logging has been enabled, this check box prompts MetaFluor to save the average ratio value for all pixels in each region of interest that has been defined.

Save Interval

Selects a saving interval for the associated wavelength or ratio image. For example, to save the image once every five acquisition cycles, select *5.* You can also set an interval of *0,* in which case the image will not be saved.

Show?

Configures MetaFluor to display the image or the image window.

Update?

Configures MetaFluor to update (refresh) the image at the interval specified by the *Update Interval* spin box.

Update Interval

Selects an image updating interval for the associated wavelength or ratio image. For example, to update the image once every five acquisition, select *5.* To update the image on every acquisition cycle, select *1.*

Calibrated Value

When data logging has been enabled, this check box prompts MetaFluor to save the average calibrated value (i.e., in user units, such as nM) for all pixels in each region in the currently calibrated image, as performed with the Acquire Calibration Standards command. The currently calibrated image will be indicated in the accompanying status text.

OK

Applies the new configuration settings and closes the dialog box.

Cancel

Disregards the new configuration settings and closes the dialog box.

Configure Ratios/FRET

Configure Ratios configures names for the ratio images and selects the wavelength images to be ratioed.

FRET performs background and bleed through correction to Fluorescence Resonance Energy Transfer (FRET) image sets.

Use the Configure Ratios command to assign the numerator and denominator wavelength images (Wavelength 1 / Wavelength 2 vs. Wavelength 4 / Wavelength 5) and a name to each of the ratio images. If you are using an external video monitor, only one ratio image will be available for configuration and display.

Note: The wavelength images are configured and named from the Configure Acquisition dialog box.

Use the FRET command to correct FRET image sets. The FRET technique involves observing the energy transfer between an excited donor fluorophore and a nearby acceptor fluorophore. This energy transfer is dependent on the overlap of excitation spectrum of the acceptor with the emission spectrum of the donor, as well as the distance between the fluorophores. Energy transfers can only occur when a donor and acceptor are very close together (within nanometers) and their spectra sufficiently overlap. Due to the overlap in spectra of the donor and acceptor, bleed through between observed wavelength channels (filter sets) influence FRET observations.

Use this command to correct FRET image sets. The FRET command will correct for background and bleed through between fluorophore filter sets using either of the following methods:

- Fully Sensitized Emission — use this method, with or without background subtraction, if you measured the bleed through of the acceptor signal and donor signal through the FRET filter set when you acquired the FRET images.

- Specified Bleed Through — use this method, with or without background subtraction, if you calibrated your system for fully specified bleed through correction; that is, you measured all possible contaminators into the FRET channel.

The following is an overview of the steps used to create a corrected FRET image in MetaFluor:

1. Acquire images of the donor, FRET, and acceptor using an appropriate filter set for each image.

2. Designate the source wavelength in the Configure Ratios/FRET dialog box.

3. Determine the FRET correction method to use — Fully Sensitized Emission or Specified Bleed Through.

4. Enter the correction constants and select the background subtraction method to be used when creating the corrected image(s).

5. Create the corrected image(s).

See Also:

Configure Ratios/FRET Procedures

Configure Acquisition

Determining FRET Coefficients

Both the Sensitized Emission and Fully Specified Bleed through methods for correcting FRET require calibration procedures. These calibrations provide the coefficients (or constants) for correcting the acquired FRET images. Perform these calibrations for each wavelength filter set used for FRET experiments. After the coefficients values are determined for each filter set, they can be used in subsequent FRET experiments.

Calibrations for both methods require controls which contain only donor fluorophore (a Donor-only control) and only acceptor fluorophore (an Acceptor-only control). These samples are used to determine bleed through between filter configurations.

Determining FRET Coefficients for Sensitized Emission

Determining FRET Coefficients for Fully Specified Bleed Through Correction

Determining FRET Coefficients - Sensitized Emission

To use the Sensitized Emission method to correct FRET image sets, you must determine values for coefficients A and B.

Coefficient A represents the degree to which the Acceptor signal is contaminating observations made in the FRET channel. This value is determined by dividing the average thresholded intensity of the image obtained using the FRET filter configuration by the average thresholded intensity of the image obtained using the Acceptor filter configuration:

$$\text{Coefficient A} = \frac{\text{Average thresholded intensity of Acceptor image from FRET filter set}}{\text{Average thresholded intensity of Acceptor image from Acceptor filter set}}$$

Coefficient B represents the degree to which the Donor signal is contaminating observations made in the FRET channel. This value is determined by dividing the average thresholded intensity of the image obtained using the FRET filter configuration by the average thresholded intensity of the image obtained using the Donor filter configuration:

$$\text{Coefficient B} = \frac{\text{Average thresholded intensity of Donor image from FRET filter set}}{\text{Average thresholded intensity of Donor image from Donor filter set}}$$

To determine the coefficient A for Sensitized Emission, complete the following procedure:

Note: This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

Step	Action
1	Use the Threshold Tool and Slider to threshold the Acceptor images acquired from both the FRET and Acceptor filter sets. **Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient.
2	Use the Spot Measurement command to determine the average thresholded intensity of both Acceptor images.
3	Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Acceptor filter set.

The result of this division is coefficient A.

To determine the coefficient B for Sensitized Emission, complete the following procedure:

Step	Action
1	Use the Threshold Tool and Slider to threshold the Donor images acquired from both the FRET and Donor filter sets. **Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient.
2	Use the Spot Measurement command to determine the average thresholded intensity of both Acceptor images.
3	Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Donor filter set. The result of this division is coefficient B.

Determining FRET Coefficients - Fully Specified Bleed Though

To use the Fully Specified Bleed Through method to correct FRET image sets, you must determine values for some or all of the following coefficients:

Donor in Acceptor

Acceptor in Donor (if applicable)

Donor in FRET

Acceptor in FRET (if applicable)

Determining FRET Coefficients - Donor in Acceptor

The Donor in Acceptor coefficient represents the degree to which signal from the Donor filter set is bleeding into observations made using the Acceptor filter set. This value is determined by dividing the average thresholded intensity of the Acceptor-only image obtained using the Donor filter set by the average thresholded intensity of the Acceptor-only image obtained using the Acceptor filter set:

$$\text{Donor in Acceptor} = \frac{\text{Average thresholded intensity of Acceptor image from Donor filter set}}{\text{Average thresholded intensity of Acceptor image from Acceptor filter set}}$$

To determine the Donor in Acceptor coefficient for Specified Bleed Through, complete the following procedure:

Note: This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

Step	Action
1	Use the Threshold Tool and Slider to threshold the Acceptor images acquired from both the Donor and Acceptor filter sets. **Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient.
2	Use the Spot Measurement command to determine the average thresholded intensity of both Acceptor images.
3	Divide the average thresholded intensity of the image obtained using the Donor filter set by the average thresholded intensity of the image obtained using the Acceptor filter set. The result of this division is the Donor in Acceptor coefficient.

Determining FRET Coefficients - Acceptor in Donor

The Acceptor in Donor coefficient represents the degree to which signal from the Acceptor filter set is bleeding into observations made using the Donor filter set. This value is determined by dividing the average thresholded intensity of the Donor-only image obtained using the Acceptor filter set by the average thresholded intensity of the Donor -only image obtained using the Donor filter set:

$$\text{Acceptor in Donor} = \frac{\text{Average thresholded intensity of Donor image from Acceptor filter set}}{\text{Average thresholded intensity of Donor image from Donor filter set}}$$

To determine the Acceptor in Donor coefficient for Specified Bleed Through, complete the following procedure:

Note: This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

Step	Action
1	Use the Threshold Tool and Slider to threshold the Donor images acquired from both the Acceptor and Donor filter sets. **Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient.
2	Use the Spot Measurement command to determine the average thresholded intensity of both Acceptor images.
3	Divide the average thresholded intensity of the image obtained using the Acceptor filter set by the average thresholded intensity of the image obtained using the Donor filter set. The result of this division is the Acceptor in Donor coefficient.

Determining FRET Coefficients - Donor in FRET

The Donor in FRET coefficient represents the degree to which signal from the Donor filter set is bleeding into observations made using the FRET filter set. This value is determined by dividing the average thresholded intensity of the Donor-only image obtained using the FRET filter set by the average thresholded intensity of the Donor -only image obtained using the Donor filter set:

$$\text{Donor in FRET} = \frac{\text{Average thresholded intensity of Donor image from FRET filter set}}{\text{Average thresholded intensity of Donor image from Donor filter set}}$$

To determine the Donor in FRET coefficient for Specified Bleed Through, complete the following procedure:

Note: This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

Step	Action
1	Use the Threshold Tool and Slider to threshold the Donor images acquired from both the FRET and Donor filter sets. **Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient.
2	Use the Spot Measurement command to determine the average thresholded intensity of both Acceptor images.
3	Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Donor filter set. The result of this division is the Donor in FRET coefficient.

Determining FRET Coefficients - Acceptor in FRET

The Acceptor in FRET coefficient represents the degree to which signal from the Acceptor filter set is bleeding into observations made using the FRET filter set. This value is determined by dividing the average thresholded intensity of the Acceptor-only image obtained using the FRET filter set by the average thresholded intensity of the Acceptor -only image obtained using the Acceptor filter set:

$$\text{Acceptor in FRET} = \frac{\text{Average thresholded intensity of Acceptor image from FRET filter set}}{\text{Average thresholded intensity of Acceptor image from Acceptor filter set}}$$

To determine the Acceptor in FRET coefficient for Specified Bleed Through, complete the following procedure:

Note: This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

Step	Action
1	Use the Threshold Tool and Slider to threshold the Acceptor images acquired from both the FRET and Acceptor filter sets. **Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient.
2	Use the Spot Measurement command to determine the average thresholded intensity of both Acceptor images.
3	Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Acceptor filter set. The result of this division is the Acceptor in FRET coefficient.

Using FRET to Correct Images - Sensitized Emission

To correct a FRET image using the Sensitized Emission method, complete the following procedure:

Step	Action
1	From the Configure menu, choose Configure Ratios/FRET. The Configure Ratios dialog box will appear.
2	Select *FRET* from the *Image and data calculations* field.
3	Select *Sensitized Emission* from the FRET Method field.
4	Select the desired donor, acceptor, and raw FRET images using the *Donor, Acceptor, and Raw FRET* image selectors
5	Enter values for *Constant A* and *Constant B*.
6	Select the desired FRET result from the *FRET result* field.
7	Click *OK* to create a correct FRET image using the settings you entered.
	OR
	Click *Cancel* to exit the FRET dialog box without creating a corrected image.

Using FRET to Correct Images - Specified Bleed Through

To correct a FRET image using the Specified Bleed Through method, complete the following procedure:

Step	Action
1	From the Configure menu, choose Configure Ratios/FRET. The Configure Ratios dialog box will appear.
2	Select *FRET* from the *Image and data calculations* field.
3	Select *Specified Bleed Through* from the FRET Method field.
4	If you have a donor image but no acceptor image, select *Donor Only* from the FRET Method field. OR If you have both a donor and an acceptor image, select *Donor and Acceptor* from the FRET Method field.
5	Select the desired donor, acceptor (if applicable), and raw FRET wavelengths using the *Donor, Acceptor, and Raw FRET* image selectors.
6	Enter bleed through coefficient values for the following fields: • *Donor in Acceptor* • *Acceptor in Donor* (if applicable) • *Donor in FRET* • *Acceptor in FRET* (if applicable)
7	Select the desired FRET result from the *FRET result* field.
8	Click *OK* to create a correct FRET image using the settings you entered. OR Click *Cancel* to exit the FRET dialog box without creating a corrected image.

Configuring Ratios

To configure ratios, use the following procedure:

Step	Action
1	From the Configure menu, choose Configure Ratios/FRET. The Configure Ratios dialog box will appear.
2	Select Ratio from the Image and data calculations field.
3	From the *Ratios to Configure* list, select the ratio you want to assign: *Ratio 1* will configure the Wavelength 1 / Wavelength 2 ratio (this will be the only ratio available if you are using an external video monitor), and *Ratio 2* will configure the Wavelength 4 / Wavelength 5 ratio.
4	If needed, type a new name for the ratio image in the *Ratio Name* text box.
5	Repeat Steps 3 and 4 as necessary for the second ratio image.
6	When you have finished, click *OK.*

Configure Ratios/FRET - Dialog Box Options

Image and data calculations

Selects which calculation to perform — select either a ratio or FRET calculation.

Ratio to Configure

Selects the ratio *(Ratio 1* or *Ratio 2)* to be configured.

Ratio Name

Assigns a name to the ratio image associated with the ratio selected from the *Ratio to Configure* list. The default names are *Ratio* and *Ratio 2.*

Ratio Calculation

This status text displays the equation for the ratio selected from the *Ratio to Configure* list, indicating which wavelength image will be the numerator and which will be the denominator. This field is only available when Ratio is selected in the *Image and data calculation* field.

FRET Method

Sensitized Emission

Uses a simplified formation of the fully specified bleed through correction method. It corrects for bleed through from the donor and acceptor into the FRET channel.

Specified Bleed Through

Use this method if you calibrated your system for fully specified bleed through correction; that is, you measured all possible contaminators into the FRET channel.

Donor Only

Enables you to select the Donor and Raw FRET source images or stacks. This option is only available if *Specified Bleed Through* is selected in the *Source* field.

Donor and Acceptor

Enables you to select the Donor, Acceptor, and Raw FRET source images or stacks. This option is only available if *Specified Bleed Through* is selected in the *Source* field.

Donor

Selects the Donor image.

Acceptor

Selects the Acceptor image.

Raw FRET

Selects the raw FRET image.

Constant A

Enables you to select the value of Coefficient A. This option is only enabled when *Sensitized emission* is selected in the FRET method field.

Constant B

Enables you to select the value of Coefficient B. This option is only enabled when *Sensitized emission* is selected in the FRET method field.

Donor in Acceptor

Selects the bleed through coefficient of the donor wavelength through the acceptor filter set.

Acceptor in Donor

Selects the bleed through coefficient of the acceptor wavelength through the donor filter set. This option is only enabled if *Donor and Acceptor* is selected in the *FRET Method* field.

Donor in FRET

Selects the bleed through coefficient of the donor signal in the raw FRET image.

Acceptor in FRET

Selects the bleed through coefficient of the acceptor signal in the raw FRET image. This option is only enabled if *Donor and Acceptor* is selected in the *FRET Method* field.

FRET result

Corrected FRET intensity

Resulting image contains the corrected FRET intensity.

Ratio of corrected FRET to Donor

Resulting image contains ratio of corrected FRET to donor.

OK

Applies the new ratio image configuration or FRET settings and closes the dialog box.

Cancel

Cancels the command and closes the dialog box.

Z Position Control (Configure Menu)

Adjusts the Z position (focus) of the objective using the selected Z-motor and provides access to the Z motor calibrations and speed setting.

Use this command to manually control the Z-motor to focus the microscope and to specify the Z-motor settings needed to enable efficient auto focusing of the microscope. The Z Position Control enables you to select the Z-motor that you want to use to focus, change the Z-motor position, set the Z origin, move the objective to the Z origin, specify the step increment size, define a custom Z step increment, calibrate the Z motor, and set the Z motor speed.

Controlling Z Position

To set and/or control your Z position settings, complete the following procedure.

Step	Action
1	From the *Configure* menu, click *Z Position Control* the Z Position Control dialog box opens.
2	In the *Z Motor MetaDevice* box, select the Z-motor that you want to use.
3	Click *Calibrate Motor*. The **Z-Axis Calibration** dialog box opens. Complete the steps to calibrate your Z motor, then return to this dialog box.
4	Click *Set Motor Speed*. The *Set Motor Speed* dialog box opens. Move the slider control to set the Z motor speed (if applicable), then click *Close* to return to this dialog box.
5	Open the *Z Step Increment* drop-down box, select the Z step increment that you want to use, or select *Custom* and type the increment value in the adjacent box. The increment value you specify appears on the Z position control arrow buttons.
6	Click *Poll Z Position* to instruct MetaFluor to obtain Z position information from the Z-motor controller.
7	Click *Enable Z Move for each wavelength* to allow each wavelength to have a separate Z position.
8	Click the *Up* or *Down* arrows to manually move the Z position by the step increment displayed on the arrow button. Changing the Z Step Increment value changes the value shown on these buttons.
9	Click *Set Origin* to set the Z origin to the currently displayed Z position in the Z box. After you click Set Origin, the Z box will indicate zero.
10	Click *Goto 0* to move the Z-motor to the origin (zero) position.
11	Click *Close* to close the Z Position control dialog box.

Z Position Control - Dialog box options

Z Motor

Indicates the name of the selected Z motor and the user-assigned unit of measure in parentheses.

Z:

Indicates the current Z position in the user-specified unit of measure.

Set Origin

Sets the current Z position to the origin (zero) position.

Less<<

Minimizes the dialog box to show only the essential controls for manually controlling the Z position (focus), setting the Z origin, and moving the Z position directly to the zero position.

More>>

Expands the dialog box to make available controls for selecting the Z-Motor, specifying the Z Step Increment, requesting the command to Poll the Z Position, enabling Z movement for each wavelength, calibrating the Z-motor, and setting the Z-motor speed.

(Up Arrow)

Moves the Z position up by one Z Step Increment for each button click.

Goto 0

Returns the Z-motor position to the zero (origin) position.

(Down Arrow)

Moves the Z position down by one Z Step Increment for each button click.

Close

Closes the Z Position Control dialog box.

Poll Z Position

Request the command to obtain the Z Position information from the Z motor.

Enable Z Move for each wavelength

Enables each wavelength to have a different Z position.

Z Motor MetaDevice

Specifies the Z-motor metadevice that you are using.

Z Step Increment

Specifies the size of the Z Step Increment. Select .1, .5, 1.0, 5.0, 10.0, or custom to specify a custom value in the adjacent box.

Calibrate Motor

Opens the *Calibrate Motor* dialog box.

Set Motor Speed

Opens the *Set Motor Speed* dialog box if your Z motor provides variable speed settings. After the *Set Motor Speed* dialog box opens, move the slider control to change the motor speed.

Note: If your Z motor is not variable speed, the *Set Motor Speed* button will be inactive.

Z-Axis Calibration

Specifies the formula for converting device units to your choice for user-units and calibrating the Z motor control to the selected user unit of measure.

Use this dialog box to specify the user unit of measure that you want to apply to the Z axis and the calibration value that you want to apply to the Z motor. This dialog box indicates the device unit name assigned to the Z motor device. You can specify your own unit name and designate an equivalency value. In addition, you can specify an appropriate unit name that you want to display. For example, you can designate microns as the unit name and specify that one micron equals four steps.

Calibrating the Z-Axis

The following procedure assumes that you have established the origin (zero) position in the Z position Control dialog box.

To calibrate the Z-axis position and specify the User Unit name and equivalency value, complete the following procedure.

Step	Action
1	From the *Z Position Control* dialog box, click Calibrate Motor. The Z-axis calibration dialog box opens. The initial Z-axis position and device units value should be set to zero.
2	Select the Step Count multiplier value that you want to apply to the Z-axis position value. The value will be displayed on both the Z-axis control buttons.
3	Click the either the positive (F6) or negative (F5) Z-Axis Control button to add the value on the button to the Z-Axis position. The Z-motor will change position whenever the Position value is changed.
4	Change the Step Count multiplier values to obtain a new value to apply to the Position value, then click the appropriate plus or minus button to add the value displayed on the button to the *Position* value.
5	When you have set the displayed Position value to a value that you want to use for the device unit value, click the *Use* button. OR Type the value that you want to use as the Device Units value.
6	Type the Name that you want to use for the user units name in the *User Units Name* box.
7	Type the User Units value that you want to use in the *User Units* box.
8	Click *Reset* to reset the Z-axis position value and the actual Z-motor position to zero.
9	Click *OK* to apply your settings and close the dialog box OR Click *Close* to disregard the settings, and close the dialog box.

Z-Axis Calibration - Dialog Box Options

Units Conversion Equation

Enables you to specify the User Units and the User Unit name in conjunction with a specific Device Units Name.

User Units

Specifies the user unit value to equate to the specified Device Unit value. This value can be a whole number or a decimal.

User Units Name

Specifies the name of the unit of measure that you want to use. This value can be a whole number or a decimal.

Device Units

Specifies the Device Unit value that is to be equated to the User Unit value.

Device Units Name

Indicates the Device Unit name as specified by the device driver.

Z Axis Control

Changes the Z-Axis (focus) position, and indicates the Z-axis position value for the current Z-axis position.

Reset

Resets the Position value to zero.

+<Value> (F6)

Adds the value displayed on the button to the position value. By changing the Step Count value and clicking this button multiple times, you can obtain and set precise Z-axis position values.

Position

Indicates the current Z-axis position value.

<– Use

Applies the currently displayed position value as the Device Units value.

-<Value> (F5)

Subtracts the value displayed on the button from the position value. By changing the Step Count value and clicking this button multiple times, you can obtain and set precise Z-axis position values.

Step Count

Select the decimal multiplier to apply to the Z-axis position value indicated as *Position*. When you select a different multiplier, the value shown on the (F5) and (F6) buttons changes to reflect the selected value.

Configure Dual View

Separates multiple wavelength images that have been acquired using an emission splitter device and a single camera. Acquired images can be oriented either vertically or horizontally, depending on the orientation of the image splitter, and are derived from two separate wavelengths of the same sample projected onto a single camera chip.

Drop-in: DUALVIEW

Use this drop-in to separate and organize multiple wavelength images of a single sample originally acquired as one image using a single camera. When the images are separated, they can be overlaid and combined into a single image composed of each individual wavelength assigned to a discrete representative color.

The advantage of using an image splitter is to enable you to simultaneously acquire two or four images of the same sample at different wavelengths. Each image is identical to the others and each can be acquired using a different emission filter. The images can then have appropriate overlay colors assigned to make it easier to identify the separate wavelengths.

Note: To use the Dual View command, you must first open the preferences dialog box on the File menu, select the Dual View tab, and click *A DualView Emission Splitter is installed.* In the *Orientation* box, you should also specify whether your emission splitter is oriented vertically or horizontally.

Configuring Dual View

To configure your dual view settings, complete the following procedure:

Step	Options
1	If you have not already done so, open the preferences dialog box on the File menu, select the Dual View tab, and click *A DualView Emission Splitter is installed.* In the *Orientation* box, you should also specify whether your emission splitter is oriented vertically or horizontally. This will enable you to configure the Dual View command.
2	With a project open, choose *Configure Dual View* from the Configure menu. The Configure Dual View dialog box opens.
3	To change the size of the image acquisition area for both wavelengths, click *New Size.* The New Size dialog box opens.
4	In the New Size dialog box, type or select the size of the image acquisition area that you want to use. The size values that you type will be applied to both wavelengths.
5	In the New Size dialog box, click OK to apply the new size values and close the dialog box. When the dialog box closes, the boxes in the image windows that represent the image acquisition areas will change to reflect the changes that you made in the New Size dialog box.
6	To change the locations of each acquisition area for each wavelength, type or select the appropriate X/Y coordinate values in the X and Y boxes. **OR** You can interactively position the acquisition areas using your mouse. Click and drag each acquisition area until it is correctly positioned.
7	Click the *Locked* check box to lock the positions of both acquisition areas.
8	When you have finished making your settings, click *Closed.*

Configure Dual View - Dialog Box Options

Wavelength 1

Specifies the X/Y coordinates of the top and left edges of the frame for wavelength 1.

X

> Specifies the X coordinate of the left edge of the frame for wavelength 1.

Y

> Specifies the Y coordinate of the top edge of the frame for wavelength 1.

Wavelength 2

Specifies the X/Y coordinates of the top and left edges of the frame for wavelength 2.

X

> Specifies the X coordinate of the left edge of the frame for wavelength 2.

Y

> Specifies the Y coordinate of the top edge of the frame for wavelength 2.

(Image Windows)

Indicates the relative position of the acquisition areas for each wavelength within the defined area of the camera chip. Click and drag each acquisition area box to interactively change the position of each box.

Image Size

Indicates the dimensions of the acquisition area for each wavelength. The acquisition area can be changed using the *New Size* dialog box; however, this area will be the same size for both acquisition areas.

Camera Area

Indicates the dimensions of the total camera area. These values represent the total area of the camera chip, and do not change when you change the size of the acquisition areas.

Locked

Locks or unlocks the acquisition area positions for both wavelengths.

New Size

Opens the *New Size* dialog box. Use the settings in this dialog box to specify the dimensions of the acquisition area for each wavelength. Changing these values changes size of the acquisition area for both wavelengths at the same time.

Close

Closes the Configure Dual View dialog box.

Image Display Controls - Using the Computer's Monitor (Configure Menu)

Sets the thresholds, display mode, digital contrast settings, ratio limits, and ratio display for the experiment's images.

Use this command to set separate **threshold levels** and **digital contrast settings** for each wavelength image. You can select either the **Monochrome or Pseudocolor display mode** for the wavelength images. For the ratio images, you can select the minimum and maximum ratio limits and the image display. You can use the Monochrome, Pseudocolor, or **Intensity Modulated Display** (IMD) display mode for the ratio images.

CAUTION:
If you are acquiring 16-bit images and wish to adjust the contrast and brightness, you must use the **Scale 16-Bit Images** command (Run Experiment menu) to do so. Because of the far greater number of intensity levels in a 16-bit image, the effects of merely adjusting the contrast settings from the Image Display Controls dialog box may have little visible effect on the image display. In addition to adjusting image contrast and brightness, the Scale 16-Bit Images command allows you to scale a selected range of 16-bit intensity levels to a 256-level intensity display, thereby greatly increasing the effect of the contrast adjustments.

See Also:

Image Display Controls - Using an External Video Monitor

Scale 16-Bit Images

Image Display Controls - Using an External Video Monitor (Configure Menu)

Sets the thresholds, display mode, ratio limits, and ratio display for the experiment's images.

Use this command to set separate **threshold levels** for each wavelength image. You can select either the **Monochrome or Pseudocolor display mode** for the wavelength images. For the ratio, you can select the minimum and maximum ratio limits and the display used for the ratio. You can use the Monochrome, Pseudocolor, or **Intensity Modulated Display** (IMD) mode for the ratio.

See Also:

Image Display Controls - Using the Computer's Monitor

Setting the Image Display Controls for Wavelength Images

To set the image display controls for wavelength images displayed on the computer's monitor, use the following procedure. **(Note:** To adjust the contrast in the display of a 16-bit image, you must use the Scale 16-Bit Images command.)

Step	Action
1	From the Configure menu, choose Image Display Controls. The Image Display Controls dialog box will appear.
2	From the *Window* list, select the wavelength image for which you want to change the image display settings.
3	Select the display mode you want to use for this image from the *Display* list.
4	Adjust the *Contrast* slider to a value that optimizes the appearance of the image.
	The default value is *50* (unmodified contrast) while maximum contrast is *100* (which produces a binary image). Contrast can not be decreased.
5	Adjust the *Brightness* slider to a value that is suitable for the selected contrast.
	The default value is *50.* Minimum brightness is *0,* while maximum brightness is *100.*
	Note: Adjust *Brightness* and *Contrast* together until you have enhanced the image to suit your viewing needs.
6	Use the *Low Thresh* and *High Thresh* sliders to set a threshold range that excludes gray levels containing areas/objects that are not of interest. As you change the threshold levels, MetaFluor will place a black overlay over the areas that are excluded.
	Note: If you can't see the black overlay, click the Threshold Tool in the image window toolbar so that the black "T" changes to red. This will enable thresholding.
7	Acquire a set of images using the Experiment Control Panel. If the threshold or ratio settings are not appropriate, repeat Steps 4 and 5.
8	Choose *Close* when you have finished setting the image display controls, or see **Setting the Image Display Controls for Ratio Images** to adjust the display of ratio images.

Setting the Image Display Controls for an External Video Monitor

To set the image display controls for an external video monitor, use the following procedure:

Step	Action
1	From the Configure menu, choose Image Display Controls. The Image Display Controls dialog box will appear.
2	Select either *Monochrome* or *Pseudocolor* from the *Image Display Mode* list as the display mode of the wavelength images.
3	Select the ratio limits appropriate for your experiment using the *Minimum* and *Maximum* spin boxes. The maximum ratio should be slightly larger than the maximum value you expect to observe during the experiment. **Note:** The ratio settings will take effect when the first pair of images is acquired after you choose *Apply.*
4	Select the display for the ratio using the *Ratio Display* drop-down list. You can select *Monochrome, Pseudocolor,* or one of the IMD displays. Your choice for an IMD display will depend on whether you expect the ratio image's values to be evenly distributed throughout the ratio range or clustered around one ratio value. If most of the values are clustered, *4 Ratios with 64 Intensities* will produce the best results.
5	If you selected one of the IMD display options in Step 4, use *IMD Intensity* to select which wavelength image is to be used to determine the intensity component of the display. You can select the intensity of either the numerator wavelength image, the intensity of the denominator image, or an average from the two images. **Note:** You should select the intensity from the brighter image. If you are not sure, you can select *Average Num. and Denom.* to use an average from the two images.
6	To set the threshold levels for Wavelength 1, select *Wavelength 1* from the *Image* group. Then set a threshold range using the slider or the *Low* and *High* text boxes. **Note:** The threshold settings will take effect when the first pair of images is acquired after you choose *Apply.*
7	Set the threshold levels for the other wavelength images, as necessary, by

repeating Step 6.

8 Choose *Apply.*

9 Acquire one set of images using the Experiment Control Panel. If the threshold settings are not appropriate, repeat Steps 6 and 7.

10 Choose *Close.*

Setting the Image Display Controls for Ratio Images

To set the image display controls for ratio images displayed on the computer's monitor, use the following procedure:

Step	Action
1	If the Image Display Controls dialog box is not already open, choose Image Display Controls from the Configure menu.
2	From the *Window* list, select the ratio image for which you want to change the image display settings.
3	Select the display mode you want to use for this image from the *Display* list. You can select *Monochrome, Pseudocolor,* or *IMD Display.*
4	If you selected *Monochrome* or *Pseudocolor* in Step 3, proceed to Step 5. **OR** If you selected *IMD Display,* skip to Step 8.
5	Adjust the *Contrast* slider to a value that optimizes the appearance of the image. The default value is *50* (unmodified contrast) while maximum contrast is *100* (which produces a binary image). Contrast can not be decreased.
6	Adjust the *Brightness* slider to a value that is suitable for the selected contrast. The default value is *50.* Minimum brightness is *0,* while maximum brightness is *100.* **Note:** Adjust *Brightness* and *Contrast* together until you have enhanced the image to suit your viewing needs.
7	Use the *Min. Ratio* and *Max. Ratio* sliders to select the ratio limits that are appropriate for your experiment. The maximum ratio should be slightly larger than the maximum ratio you expect to observe during the experiment. Now jump to Step 11.
8	If the display mode is *IMD Display* and you are displaying ratio images as 8-bit images (Preferences : General command, File menu), the *Brightness* and *Contrast* sliders will have changed to *IMD Display* and *IMD Intensity* drop-down lists. Select the desired IMD mode from the *IMD Display* drop-down list: *4 Ratios with 64 Intensities, 8 Ratios with 32 Intensities,*

16 Ratios with 16 Intensities,
32 Ratios with 8 Intensities, or
64 Ratios with 4 Intensities.

9 If the display mode is *IMD Display* and you are displaying ratio images as 24-bit images, the *Brightness* and *Contrast* sliders will have changed to *IMD Intensity* and *IMD Overlay* drop-down lists.

Use the *IMD Overlay* drop-down list to select the wavelength image that will be used to determine the saturation of the color component of the ratio image. If you select *None,* maximum saturation will be used.

10 Use the *IMD Intensity* drop-down list to select which wavelength is to be used to determine the intensity component of the display. You can select the intensity from either wavelength or an average from the two wavelengths.

If you are using the 24-bit ratio image display, you also have the option of selecting either the mean intensity *(Average of Wavelengths)* or the maximum intensity *(Maximum of Wavelengths)* of all wavelength images as the intensity component.

11 Acquire a set of images using the Experiment Control Panel. If the settings are not appropriate, repeat Steps 4 - 7 (Monochrome or Pseudocolor) or Steps 8 - 10 (IMD Display), as needed.

12 Choose *Close* when you have finished setting the image display controls.

Measure Spectra (Configure Menu)

Measures a dye's response using your microscope and illumination devices, so that you can select the best excitation wavelength for your experiment.

This command is used as a diagnostic tool when setting up new illumination devices. For example, you may find that your equipment works better with a slightly different wavelength from one that has been published for a particular dye.

Note: You must install a continuous wavelength Illumination MetaDevice, such as a monochromator, before using the Measure Spectra command.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Configure Illumination

Define Regions for Measurement

Measuring Spectra

To measure spectra, use the following procedure. **Note:** You must have at least one region of interest defined in the image window.

Step	Action
1	From the Configure menu, choose Measure Spectra. The Measure Spectra dialog box will appear.
2	From the *Configure Wavelengths* drop-down list, select the Illumination MetaDevice that you want to use for the measurement procedure. **Note:** You must select a continuous wavelength illumination device, such as a monochromator.
3	Select the first wavelength to be measured using *Starting Wavelength.* Then select the last wavelength to be measured using *Ending Wavelength.*
4	Select the increment to be skipped between measured wavelengths using *Step Wavelengths By.* Select *1* as the increment if you want to measure every wavelength.
5	To use a particular set of wavelength image acquisition settings, select the wavelength image from the *Use Acquisition Settings of Wavelength* drop-down list. Otherwise, just select *(Use Current).*
6	If your camera requires a delay, enter the delay value in the *Delay (ms) to Wait After Stepping Wavelength* text box. If you are leaving the shutter open, you will want to enter your camera lag here.
7	If you want the shutter to be closed between acquisitions, select the *Open Shutter Before Each Measurement, Then Close It* check box. **OR** If you want the shutter to stay open during the entire acquisition process, leave the check box cleared.
8	If you want to use one of the defined regions to provide an average intensity value that will be subtracted as a background value from the other acquisition regions, select the region from the *Bkgd Region* drop-down list. Otherwise select *None.*
9	From the *Regions to Measure* table, double-click the entries for the regions that you want to measure. A check mark will appear next to each selected entry.
10	When you are ready, choose *Measure.* The

spectral measurement(s) will be displayed in the Spectra graph.

11 If you want to save the results of the measurement procedure (wavelength and associated average intensity level), choose *Save Last Set of Measurements.* The Export dialog box will appear. Choose

Save Info to a Text File to save the data as a .txt file,

Copy Info to the Clipboard to copy the data to the Windows environment's Clipboard (you can then paste the data into another Windows-based program, such as a word processor), or

Print Info to send the data to the default printer.

12 From the Measure Spectra dialog box, choose *Close* when you have finished.

Measure Spectra - Dialog Box Options

Configure Wavelengths

Specifies the Illumination MetaDevice used for this command from the Illumination MetaDevices currently available. **Note:** You must select a continuous wavelength illumination device, such as a monochromator.

Starting Wavelength

Specifies the first wavelength to be measured.

Ending Wavelength

Specifies the last wavelength to be measured.

Step Wavelengths By

Selects the increment to be skipped between measured wavelengths. Select *1* as the increment if you want to measure every wavelength.

Use Acquisition Settings of Wavelength

Configures the spectral measurement acquisition to use the acquisition settings of the selected wavelength image.

Delay (ms) to Wait After Stepping Wavelength

Specifies a camera delay between the changing of the wavelength and acquisition of the measurement image.

Open Shutter Before Each Measurement, Then Close It

When selected, this check box configures the shutter to close between acquisitions. When this check box is left cleared, the shutter will remain open during the entire measurement procedure.

Bkgd Region

Selects a defined region of interest to use for background subtraction. The average intensity value in this region will be subtracted from the average value of each spectral measurement region.

Regions to Measure

Lists all defined regions of interest. Select the regions that you want to measure by double-clicking the corresponding entries. A check mark will appear next to each selected entry. To deselect an entry, double-click it again.

Measure

Starts the spectral measurement procedure, displaying the results in the Spectra graph.

Save Last Set of Measurements

Opens the Export dialog box. From this dialog box, you can save the measurement data as a text (*.txt) file, copy it to the Clipboard, or send it to the default printer.

Spectra Graph

Displays the spectral measurement(s). This graph can be configured by clicking the Down Arrow button and choosing the appropriate command from the configuration menu that appears.

Close

Closes the dialog box

Configure Illumination (Configure Menu)

The Configure Illumination command enables you to create unique settings for the hardware devices used to control illumination in MetaFluor.

Use the Configure Illumination command to create settings for multiple hardware devices that affect illumination. Once created, the positions of these devices are saved as one illumination setting that can be automatically applied in MetaFluor.

The following aspects can be modified and saved using the Configure Illumination command:

- Open and close shutters
- Set the wavelength and intensity of the illumination by controlling the appropriate window.
- Set the wavelength value for images.

Note: The number of device controls available will vary according to your hardware configuration.

Before you use the Illumination command, you will need to install and configure the appropriate hardware device driver(s) using the Meta Imaging Series Administrator.

WARNING:
Turn off all electronics, including computers, before powering up your arc lamp light source. The electromagnetic pulse (EMP) generated by arc lamp (mercury or xenon) starters can damage any electronics near the power supply, lamp house, or cables that connect the power supply and lamp.

WARNING:
You must run all mechanical shutters at a cycle time greater than 25 ms. Uniblitz, Lambda 10, Metaltek, Ludl, and cooled CCD shutters are driven by a high voltage which takes time to dissipate. Running these shutters at a cycle length shorter than 25 ms will cause a build-up of heat, leading to eventual jamming. Neither Molecular Devices nor any manufacturers of the aforementioned shutters will honor warranties on equipment that has been damaged by improper use. Operation of these shutters at a cycle length shorter than 25 ms will be considered improper use.

Creating Illumination Settings

The options that appear in the Configure Illumination dialog box vary based on your installed hardware. To create a new illumination setting, use the following procedure:

Step	Action
1	With a project open, from the Configure menu, click *Illumination Control.* The Configure Illumination dialog box opens.
2	Type a name for the new setting in the *Name* field.
3	To specify a wavelength value for images acquired using this setting, type or select a value from the *Wavelength* field. The color box displays the LUT for the wavelength value you enter.
4	To select the active filter for a filter wheel, click the check box next to the filter wheel name and select the filter with the slider.
5	If you have a monochromater, click the check box next to the monochrometer name and use the slider or edit box to select the wavelength to be used during acquisitions.
6	To change the state of a shutter, click the checkbox next to the shutter name and select *Always Closed* or *Active.*
7	To manually check the shutter positions for a setting, click the *Open/Close Shutter* icon.
8	To open the shutter when the setting is active, click *Open shutters when setting is selected.* This is done primarily when you are using transmitted light and bleaching and photo damage is not a concern. When this option is selected the shutters will not open or close for acquisitions because they will be in their proper state as soon as a setting becomes active.
9	Click *Add/Replace* when your setting is complete. The new setting name is displayed in the *Defined Settings* field and the setting is saved.
10	Click *Close.*

Deleting Illumination Settings

To delete an existing illumination setting, use the following procedure:

Step	Action
1	From the Devices menu, click Illumination. The Configuring Illumination dialog box opens.
2	Click the name of the setting you want to delete in the *Defined Settings* field.
3	Click *Remove.*
4	Click *Close.*

Configure Illumination - Dialog Box Options

Name

Specifies a new illumination setting name when you add a setting with *Add/Replace*.

Wavelength

Specifies wavelength value for the illumination setting. This value is stored in images created using this illumination setting. The color box shows the LUT that corresponds with the wavelength. If you set the wavelength outside the visible range (380-780), the color box turns white.

Defined Settings

Lists currently available defined illumination settings. Double click a setting to make it active. The settings in the dialog box will change to reflect the new setting.

Open shutters when setting is selected

Determines if shutters set to open for each illumination setting do so when you select a setting. If not selected, shutters will open only during acquisition. Leave this box unchecked to use the Open/Close Shutter icon to verify your shutter settings.

Open/Close Shutter

Opens and closes shutters as defined by the active illumination setting. This command is useful if you want to verify the correct shutters are responding for a setting.

Device Positions

Lists configurable settings:

> **Note**: The type and number of settings will vary based on your hardware configuration.

> **Filter Wheel** – Select the active filter for each filter wheel. If you have a monochromatic device you can enter the number in the box.

> **Shutter** – Select Closed or Open for each shutter. This is the state you want the shutter to be in during acquisition. If the *Open shutters when setting is selected box* is checked, then this will be the state the shutter will be in when the setting is selected.

Monochromater – Click the check box next to the Monochromater name and use the slider or edit box to select the wavelength to be used during acquisitions.

Add/Replace

Adds new or edits existing defined settings.

Remove

Deletes the setting selected in the Defined Settings list.

Setting that defines shutter for command bar "Shutter" button

Designates the name of the defined setting that will appear on the shutter command bar button. Open the drop-down list and choose the setting name that you want to use.

Backup

Opens the *Backup all Illumination settings* dialog box. Use this command to save your current Illumination settings to a file. These settings are saved to a .ILS file. The settings can be restored and reused later. Each time you change illumination settings, you can back them up. Then if you need to return to a previous illumination configuration, you can restore the settings and reuse them without having to redo them.

Restore

Opens the *Restore Illumination settings* dialog box. Use this command to retrieve a previously backed-up illumination settings file to restore a previously used and saved group of illumination settings.

Close

Closes the dialog box.

Run Experiment Menu

Experiment Control Panel - Digital Camera (Run Experiment Menu)

Contains the focusing and image and data acquisition commands needed to run a new experiment using a digital camera.

The Experiment Control Panel is your "command center" for controlling new experiments. Using it, you can focus the camera, set image acquisition settings, and enable image saving and data logging. If your camera has gain and offset controls, you can adjust these while focusing the camera.

You can use the *Focus* command button to obtain continuous images while focusing the microscope prior to starting the experiment. As you focus the microscope, MetaFluor continuously acquires and displays images which you can use to verify that your specimen is visible and in focus. It is important to use the *Focus* command while you are focusing the microscope, because what can be seen through the microscope's eyepiece is not always the same as what the camera acquires.

If your camera supports it, you will have the option of using an external video display or an image window on your computer screen to view the focusing image. You can configure your choice by selecting a preference in the Digital Camera Preferences dialog box (Preferences command, File menu). Digital cameras that currently support this flexibility of focusing methods include the Princeton Instruments PentaMax and MicroMax. If you use an external video monitor, the *Focus* command will summon the Focus Digital Camera dialog box, which you can use to zoom and pan, set the excitation wavelength and exposure time, and specify an intensity scaling and gray level offset for the focus image. If you use an image window, the *Focus* command will summon the Focus Control dialog box, which specifies the pixel binning, exposure time, and focusing region for the focusing image. In both cases, these focusing options are different from those used for actual data image acquisition, which is configured using the **Configure Acquisition** command.

The majority of the commands in the Experiment Control Panel are image and data acquisition commands. Most are used prior to starting an experiment to define the acquisition. "LED" indicators next to the *Save Images* and *Save Ratios* check boxes will indicate the saving status of each Wavelength and Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Wavelength or Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle. When you have selected all of the necessary options, the experiment can be run. You can enable Background subtraction and shading correction using the **Reference Images** command.

Before you start acquisition, spend a minute to make sure that **Image Display Controls** and **Configure Experiment** are set appropriately for your experiment. You may also want to check the Data Logging Preferences options (Preferences command, File menu) if you are logging data. If you plan to use event marks or move regions while running the experiment, you may want to open and position those dialog boxes before starting acquisition.

Note: This version of the Experiment Control Panel is unavailable in the MetaFluor Offline system. Please see the description of the **Playback Mode version.**

See Also:

Experiment Control Panel:

    For Video Camera with Computer's Monitor

    For Playback

Reference Images

Image Display Controls - Using the Computer's Monitor

Preferences

Event Marks

Focusing the Digital Camera Using a Computer Image Window

To focus the image from a digital camera using an image window on the computer screen, use the following procedure:

Step	Action
1	From the Experiment Control Panel, choose *Focus.* The Focus dialog box will appear. MetaFluor will temporarily close all other dialog boxes that are open.
2	From the *Wavelength* table on the right side of the dialog box, select the wavelength image you want to use for focusing.
3	If your camera supports pixel binning, use the *Binning* spin box to select a binning value. This value will be used for binning in both the vertical and horizontal directions.
4	From the *Exposure Time* spin box, select the acquisition exposure time for the focusing images. **AND** If your camera supports them, select a camera gain from the *Gain* list, select a camera bit-depth from the *Bits* list, and select a transfer speed from the *Rate* list. If your camera supports on-chip integration, use the *Frames to Integrate* option to select the number of frames to be summed for each acquired focal image.
5	If you changed the settings from those you configured using the Configure Acquisition command, you can direct MetaFluor to use the new settings for acquiring the data images by choosing *Store for This Wave.* **OR** If you want to use the same exposure settings as you configured for the data images with the Configure Acquisition command, choose *Use Wave Settings.*
6	If you are using an external shutter, select *Open on Start Focus.* Select *Close After Acquire* if you want the shutter to close immediately after the acquisition cycle has finished.
7	When you are ready to start acquiring focus images, choose the *Start Focusing* button. If you are using a shutter, choose *Toggle Shutter* to open the shutter. Choose it again to close the shutter. The colored box to the right of the button will indicate when the shutter is open or closed. After you choose *Start Focusing,* a new *Interactive* option group will

appear below the *Acquisition Region* option group (see Step 8, middle option).

You can pause acquisition at any time by choosing *F2: Pause Focus* or by pressing the [F2] function key. Resume focusing by choosing *F4: Resume Focus* or by pressing the [F4] function key.

8   If you want to use a subregion of the image for focusing, use the box-in-box display or the *Left, Top, Width,* and *Height* spin boxes to specify the size and location of the focusing subregion. Choose *CTR* to place the center of the region in the center of the image. Alternatively, choose *Full Chip* to use the entire image. If you want to use this newly configured region for acquisition as well, choose *Store as Acq Rgn.*

OR
Choose the *Select* button in the new *Interactive* option group that appears in the upper left area of the Focus dialog box. This will display a green region of interest on the Focus image window. Resize and reposition the region as needed with your cursor and choose *OK* to fill the entire Focus image window with the selected region.

OR
If you want to use the same acquisition region settings as you configured for the data image itself with the Configure Acquisition command, choose *Use Acq. Region.*

9   While monitoring the focusing image, adjust the microscope to provide the optimum focus.

10   If you want to view a graphical display of the intensity values in the image while adjusting the contrast, select the *Update* check box, select either *Histogram* or *Scanline* from the drop-down list, and choose the image's bit-depth from the *Depth* list.

The histogram or line graph can be configured as needed from the Down Arrow configuration menu.

11   If you are acquiring 16-bit images and want to adjust the scaling to increase contrast in the displayed images, use the *Low* and *High* text boxes or the scaling slider to select the lower and upper grayscale values, respectively, in the displayed image. Alternatively, you can drag the left (yellow) and right (cyan) calipers to define the scaling range.

OR
Select the *Autoscale* check box if you want MetaFluor to select the scaling range automatically.

12   When you have finished, toggle the shutter closed (if necessary) and choose *Stop*

Focusing.

13   Choose *Close* to return to the Experiment
Control Panel.

Focusing the Digital Camera Using an External Video Monitor

To focus the image from a digital camera using an external monitor, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel will appear.
2	Choose *Focus.* The Focus dialog box will appear.
3	With the *Exposure (ms)* spin box, select an exposure time for the focusing image.
4	If necessary, use the *Intensity Scaling* drop-down list box to select a scaling range for 16-bit focusing images.
5	If necessary, use the *Offset* spin box to specify a gray level offset for the focusing image.
6	If you are using a filter wheel or monochromator, use the *Wavelength* list to select the wavelength image corresponding to the illumination settings you want to use during image focusing.
7	When you are ready, select the *Open Shutter* check box (if you are using an external shutter), so that a check mark appears in it. **AND** Choose *Start Focusing.* Images will be acquired continuously and displayed on the external video monitor, and the progress meter will indicate the stage in the acquisition cycle.
8	Focus your microscope. As you do so, you can switch between filters using the selections in the *Wavelength* list, open or close the shutter by selecting or clearing the *Open Shutter* check box, change magnification with *Zoom,* and change the focus region with *Pan.* When you want to stop focusing, choose *Stop Focusing.*
9	When you have finished, choose *Close* to return to the Experiment Control Panel.

Preparing for Image Acquisition

To prepare for image acquisition, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel dialog box will appear.
2	Focus the microscope using the *Focus* command.
3	If you want to use background subtraction and/or shading correction, acquire the appropriate reference images and save them using the **Reference Images** command.
4	Configure the desired wavelength, intensity, MetaDevice, and shutter setting using the **Configure Acquisition** command.
5	If you want to save wavelength images, ratio images, or measured data, open the appropriate file using the **Open Save Images File, Open Save Ratios File,** or **Open Measurements File** commands.
	Note: The appropriate command will be opened automatically if you select *Save Images, Save Ratios,* or *Log Data* from the Experiment Control Panel, but a file has not yet been opened.
6	If you plan to log data, you may want to enable *Log Data* in the Experiment Control Panel now so that the location, size, and area of the regions are logged at the start of the log file.
	Do not select *Save Images* or *Save Ratios* in the Experiment Control Panel yet.
7	Use the **Define Regions for Measurement** command to define the desired regions of interest (necessary for measurements).
8	From the Experiment Control Panel, choose *Set Timelapse.* The Set Timelapse dialog box will appear.
	Select the timelapse interval and the measurement units using *Timelapse Interval.* Select *0* for no timelapse.
	Select the number of acquisitions to acquire using *Number of Acquisitions.* Select *0* if you want the acquisition to continue until you choose *Pause Acquisition.*
	Choose *Close* to return to the Experiment Control Panel.

Running the Experiment

To acquire images and/or data, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel will appear.
2	Choose *F4: Acquire* or press the [F4] function key. MetaFluor will start image acquisition.
3	Whenever you want to save wavelength or ratio images, select *Save Images* or *Save Ratios* to enable saving. Clear the *Save Images* or *Save Ratios check boxes* when you want to stop saving wavelength or ratio images. EXAMPLE: You can enable and disable *Save Images* so that images are saved from cycles 3 - 10 but not save images from cycles 1, 2, and 11.
4	You can enable or disable *Log Data* at any point during acquisition.
5	Mark events or move regions as necessary during the experiment.
6	The status line will report "Acquiring Wavelength *X*," "Transmitting Wavelength *X*," "Ratioing images," or "Next acquire in XX ms." If the acquisition time selected is shorter than the time required to complete acquisition tasks, the next acquisition will start after the previous one was finished.
7	Press the [F2] key on the keyboard or choose *F2: Pause* to stop the acquisition at any time.

Experiment Control Panel - Dialog Box Options (Digital Camera)

Status

Reports the length of time to the next acquisition, as set by *Set Timelapse* and the shutter delay time in the Illumination Control dialog box (the *Delay* option). The message "Running" occurs when an image is acquired or when the requested acquisition interval is shorter than the time required to complete the acquisition cycle.

Log Data

Enables or disables data logging to an open, DDE-linked spreadsheet file and/or text file. The status line next to *Log Data* will indicate if a file is open for saving data. If a data file has not been opened prior to selecting *Log Data,* the Open Measurements File command will be activated automatically so that you can open a file.

Save Images

Enables or disables wavelength image saving. The status line next to *Save Images* will indicate if an .inf file is open. If an .inf file has not been opened prior to selecting *Save Images,* the Open Save Images File command will be activated automatically so that you can open an .inf file. "LED" indicators next to the *Save Images* check box will indicate the saving status of each Wavelength image: gray indicates that saving is off, red indicates that saving is on but that the particular Wavelength image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle.

Save Ratios

Enables or disables ratio image saving. The status line next to *Save Ratios* will indicate when you can save ratio images. "LED" indicators next to the *Save Ratios* check box will indicate the saving status of each Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle. The Open Ratios File command will be activated automatically when you select *Save Ratios.*

F4: Acquire

Acquires wavelength image and ratios them according the display and acquisition options. When you choose this command, its label will change to *"F2: Pause."*

Set Timelapse

Sets the time between acquisitions and the number of acquisition to be completed. Select *0* for the *Timelapse Interval* if you do not want to use timelapsing. Select *0* for the *Number of Acquisitions* if you want acquisition to continue until you choose *F2: Pause.*

F2: Pause

Pauses acquisition at the end of the present cycle. You can also press the [F2] key on the keyboard to stop acquisition.

Zero Clock

Same as the Zero Clock command in the Run Experiment menu.

Focus

Opens the Focus Control dialog box if you are using an image window on a computer monitor, or the **Focus Digital Camera** dialog box if you are using an external video monitor.

Cycle and CPS

One cycle is the total time required to acquire both wavelength images (with background subtraction and/or shading correction), display them, ratio them, display the ratio image, draw regions (if selected), make measurements, plot measurements on graphs, log data, and save images. The CPS calculation is updated every 10 uninterrupted cycles.

Close

Closes the dialog box.

Focus - Dialog Box Options (Digital Camera with Computer Monitor)

Binning

Configures pixel binning for acquisition of focusing images.

Left

Defines and displays the leftmost point of the region.

Top

Defines and displays the topmost point of the region.

Width

Defines and displays the width of the region.

Height

Defines and displays the height of the region.

Use Acq Region

Directs MetaFluor to use the acquisition region settings from the Configure Acquisition dialog box for the focusing image.

Store as Acg Rgn

Directs MetaFluor to use the current focusing acquisition regions for acquisition of the data images. These settings will be stored in the Configure Acquisition dialog box.

Select (Interactive)

Displays a green region of interest on the Focus image window. After you reposition and resize the region around the area in the image that is of greatest importance, choosing *OK* will zoom that area to fill the entire Focus image window. A green "LED" will flash beneath the *OK* and *Cancel* buttons while the *Select* function is active. The *Interactive* option group *(Select, OK,* and *Cancel)* appears when you choose *Start Focusing.*

OK (Interactive)

Accepts the green region of interest drawn in the Focus image window when you choose the *Select* button, and zooms the selected area of the image to fill the entire Focus image window. The *Interactive* option group *(Select, OK,* and *Cancel)* appears when you choose *Start Focusing.*

Cancel (Interactive)

Cancels the selection of the image area selected by the green region of interest, and removes the region from the Focus image window.

<<

Decreases the size of the focusing image's acquisition subregion by half.

CTR

Centers the focusing image acquisition subregion.

>>

Doubles the size of the focusing image's acquisition subregion.

Full Chip

Specifies that the entire camera chip be used as the focusing image's acquisition subregion.

Box-in-Box Display

Allows you to define a region of interest for focusing. Drag the outline of the smaller box to resize and position it, just as you would for a data acquisition subregion. The focusing region will be defined proprotionally such that, if you change the size of the overall image (for example by changing binning) or the acquisition region, the focusing region will remain the same relative size and in the same relative location on the image.

Exposure Time

Selects an acquisition exposure time for the focusing images.

Gain

Specifies the camera gain to be used for the focusing image.

Bits

Specifies the camera bit-depth to be used for the focusing image.

Rate

Specifies the camera transfer speed of data.

Frames to Integrate

Specifies the number of frames to be summed together for each focal image. This option will appear only if your camera supports on-chip integration.

Use Wave Settings (exposure time)

Directs MetaFluor to use the camera settings from the Configure Acquisition dialog box for the focusing image.

Store for This Wave (exposure time)

Directs MetaFluor to use the current exposure time settings for acquisition of the data images. These settings will be stored in the Configure Acquisition dialog box.

Update

Enables continuous updating of the intensity values in the histogram or line graph while you configure contrast.

Histogram/Scanline Selection List

Selects a display mode for the continously updating intensity graph: *Histogram* or *Scanline.* If you select *Histogram,* the gray values in the entire image (or focus region) will be numerically represented in the histogram bins. If you select *Scanline,* you can select the location of the red scanline in the image by dragging it up or down in the image window, and the gray values under the line will be represented in a line graph.

Depth

Selects a range of intensity values for display in the intensity graph. The value should reflect the bit-depth of the camera. Select *8-Bit* for a range of 0 - 256, *10-Bit* for a range of 0 - 1024, or *12-Bit* for a range of 0 - 4096.

Intensity Graph

Displays the continuously updated intensity values in the focusing image in either a histogram or line graph.

Down Arrow Configuration Menu

Allows you to configure the intensity graph. You can also print the graph or copy it to the Clipboard. (For more information, see **Graphs).**

Wavelength

Selects a wavelength image for display of the focusing image.

Toggle Shutter

Toggles the shutter open and closed. The colored box to the right of the button will indicate when the shutter is open or closed.

Open on Start Focus

Directs MetaFluor to open the shutter at the start of the focus image's acquisition cycle.

Close After Acquire

Directs MetaFluor to close the shutter at the end of the focus image's acquisition cycle. Leave this check box cleared to perform continuous acquisition.

Low

Selects the lowest gray value to be displayed in the scaled 16-bit focusing images.

High

Selects the highest gray value to be displayed in the scaled 16-bit focusing images.

Autoscale

Directs MetaFluor to select the scaling range automatically for the 16-bit focusing images.

Use Wave Settings (scaling)

Directs MetaFluor to use the scaling settings from the Scale 16-Bit Images dialog box for the focusing image.

Store for This Wave (scaling)

Directs MetaFluor to use the current scaling settings for acquisition of the data images. These settings will be stored in the Scale 16-Bit Images dialog box.

Start Focusing / Stop Focusing

Starts and stops the acquisition of images for focusing.

F2: Pause Focus

Pauses acquisition of the focusing images.

F4: Resume Focus

Resumes acquisition of the focusing images.

Z Position

Contains settings to adjust the Z position associated with a specific wavelength and to store a newly defined Z position for the wavelength.

(Up Arrow)

Moves the Z position upward by the step size defined in the Z Position Control dialog box. The current step size is indicated on the arrow button.

(Down Arrow)

Moves the Z position downward by the step size defined in the Z Position Control dialog box. . The current step size is indicated on the arrow button.

Goto 0

Moves the Z position to the zero or origin position as defined in the Z Position Control dialog box.

Use Wave Z

Applies the Z Position settings as specified in the Z Position Control dialog box.

Store for this Wave

Stores the currently set Z Position value as the new Z position value for the currently selected wavelength, replacing any previously set Z position value for this wavelength.

Close

Closes the dialog box.

Focus Digital Camera - Dialog Box Options (Digital Camera with External Monitor)

Zoom

Selects a magnification level for the focusing image: *1x* (no zoom), *2x,* or *4x.*

Pan

If you selected a higher magnification with the *Zoom* option, this button group selects a quadrant subregion of the original image for display at the zoomed magnification.

Exposure (ms)

Specifies an exposure time, in milliseconds, for the focusing images.

Intensity Scaling

Specifies an intensity scaling range for 16-bit images. For example, if most of your image intensity data is at the lower end of the intensity grayscale, you will want to use *4 - 1024.*

Offset

Specifies a grayscale offset for the focusing image.

Wavelength

Selects the wavelength image series corresponding to the illumination settings (MetaDevice, illumination wavelength, illumination intensity, shutter usage) that you want to use for acquiring the focusing images.

Open Shutter

Toggles the external shutter open and shut. When you select this check box, the shutter will be opened.

Start Focusing/Stop Focusing

Starts and stops the acquisition of images for focusing.

Close

Closes the dialog box

Experiment Control Panel - Playback Mode (Run Experiment Menu)

Contains the playback and the image and data storage commands needed to playback a stored experiment.

When you open an experiment stored on disk using the Open Experiment command, the commands in the Experiment Control Panel change to those needed for playback, rather than for live acquisition. Using the Experiment Control Panel, you can play back the images, and, if you wish, you can log data and save wavelength and ratio images. This allows you to log data or save ratio images if you were not able to do so during the experiment. Saving images is useful if you want to selectively save a smaller set of images to another .inf file.

As during acquisition, "LED" indicators next to the *Save Images* and *Save Ratios* check boxes will indicate the saving status of each Wavelength and Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Wavelength or Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle.

See Also:

Experiment Control Panel:

   For Digital Camera

   For Video Camera with Computer's Monitor

   For Video Camera with Other Video Board and External Monitor

Playing Back a Stored Experiment

To play back an experiment, use the following procedure:

Step	Action
1	Open the desired experiment using the Open Experiment command.
2	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel will appear.
3	To view selected image frames, use the *Frame* slider, or the *F4: Forward* or *F3: Reverse* buttons. The button you click will become the *F2: Pause* button. (A second click will convert the button back to its original state as a "forward" or "reverse" button.)
	The *Frame* slider advances or reverses to a selected frame. The text box next to this slider displays the current frame. It can also be used to go to a particular frame by typing a frame number and pressing the [TAB] key.
	F4: Forward and *F3: Reverse* quickly advance the frames in the selected direction until you choose *F2: Pause* or the last frame is reached.
4	If you plan to log data, you should look for areas that would be ideal regions of interest.
	(If you made event marks while collecting images, MetaFluor will display an "Event" message box while playing back the images. After you have read the text, close the message box by pressing *Enter.)*
5	If you want to log data, or save wavelength or ratio images, use the *Frame* slider to return to Frame 1 (so that images or data are saved in the proper chronological order).
	Then select *Log Data* to enable data logging, select *Save Images* to enable wavelength image saving, or select *Save Ratios* to enable ratio image saving.
	You can deselect any of these three options at any time to disable the image saving or data logging.
6	If you plan to log data or view data using the graphs, you should define regions of interest using the **Define Regions for Measurement** command.
7	Play through images again using *F4: Forward* to log data or save wavelength or ratio images.
8	Choose *Close* when you have finished.

Experiment Control Panel - Dialog Box Options (Playback Mode)

Status

Indicates the name of the image that is currently loaded.

Log Data

Enables or disables data logging to an open, DDE-linked spreadsheet file and/or text file. The status line next to *Log Data* will indicate when a file is open for saving data. If a data file has not been opened prior to selecting *Log Data,* the Open Measurements File command will be activated automatically so that you can open a file.

Save Images

Enables or disables wavelength image saving. The status line next to *Save Images* will indicate when an .inf file is open. If an .inf file has not been opened prior to selecting *Save Images,* the Open Save Images File command will be activated automatically so that you can open an .inf file.

Save Ratios

Enables or disables ratio image saving. The status line next to *Save Ratios* will indicate when you can save ratio images. "LED" indicators next to the *Save Ratios* check box will indicate the saving status of each Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle. The Open Ratios File command will be activated automatically when you select *Save Ratios.*

Frame

Advances or reverses to the selected frame.

Image Time

Displays the image time for the current image. You can use the drop-down list to specify the units of time used to display the time.

F3: Reverse

Reverses the frames quickly until either *F2: Pause* is chosen or the first frame has been reached. When you first click this button, it will become the *F2: Pause* button. A second click will convert it back to the *F3: Reverse* button.

F4: Forward

Advances the frames quickly until either you choose *F2: Pause* or the last frame has been reached. When you first click this button, it will become the *F2: Pause* button. A second click will convert it back to the *F4: Forward* button.

F2: Pause

Pauses playback.

Close

Closes the dialog box.

Stop Acquisition (Run Experiment Menu)

Stops image acquisition during an experiment.

Use this command to stop image acquisition after the present acquisition cycle is completed.

You can stop image acquisition by any of the following methods:

(1) Choosing Stop Acquisition from the Run Experiment menu,

(2) Pressing the [F2] function key (the keyboard shortcut for the Stop Acquisition command), or

(3) Choosing *Pause* from the Experiment Control Panel.

If you keep the Experiment Control Panel open and accessible during a new experiment, choosing *Pause Acquisition* is faster than selecting Stop Acquisition from the Run Experiment menu. *Pause Acquisition* will change to *Resume Acquisition* so that you can pause and restart image acquisition with the click of a mouse button. (**Note:** Because of the way the computer communicates with its peripheral input devices, pressing the [F2] key is faster still.)

Shortcut: [F2]

See Also:

Experiment Control Panel:

For Digital Camera

For Video Camera with Computer's Monitor

For Video Camera with External Video Monitor

Stopping Image Acquisition

To stop image acquisition, use the following procedure:

Step	Action
1	Select the Run Experiment menu.
2	Choose Stop Acquisition. MetaFluor will stop image acquisition at the end of the current acquisition cycle. **Note:** You can use the [F2] key, the keyboard equivalent for the Stop Acquisition command, at any point during acquisition.

One Acquisition (Run Experiment Menu)

Acquires one set of images and then stops acquisition.

Use this command to acquire one complete acquisition cycle of images. This command provides a quick way for you to acquire one set of images for use while defining regions, selecting a save region, or setting image display controls in preparation for the experiment. If you are playing back an experiment, this command will not be available.

The One Acquisition command has a keyboard shortcut, the [F3] function key, which you can use rather than selecting the command from the menu. For example, you can use the shortcut while determining the threshold settings for a new experiment in the Image Display Controls dialog box. If you press the [F3] key after choosing *Apply* from the Image Display Controls dialog box, the images on your monitor will be updated with the new settings without leaving that dialog box.

There is an *Acquire Images* command button in the Select Source Image for Editing Regions dialog box (Define Regions for Measurement command, Graphs menu). You will not be able to use the [F3] keyboard shortcut when this dialog box for selecting source images is open.

Shortcut: [F3]

Acquiring One Acquisition

To acquire one set of images, use the following procedure:

Step	Action
1	Select the Run Experiment menu.
2	Choose One Acquisition. MetaFluor will acquire one set of images.

Zero Clock (Run Experiment Menu)

Resets the clock used for graphing and logging data and clears any data in the graphs.

Use this command to reset the clock used to graph and log data for a new experiment to its original starting point. Any data graphed in the measurements graphs will be cleared when you use the Zero Clock command. If data logging has been enabled, the message "Clock reset to 0.0" will appear in the log file. This command is ideal for synchronizing all of your data-gathering equipment.

Because the clock restarts immediately, you should arrange everything for image acquisition before using Zero Clock. (When you start a new experiment, the clock will not start until the first image is acquired.)

Understanding the Clock

MetaFluor uses real-time units (seconds) to present acquired data in data logs and graphs. When you start image acquisition for a new experiment, the clock starts at 0.0. If you started image acquisition at 3:30 PM, the "0.0 seconds" at the start of the graphs and at the first entry in the log file would refer to 3:30 PM. If a point of data was plotted at 1372 seconds on the graph, that would reflect that something happened to the experiment at 3:52:52 PM. This clock is ideal for representing relative times; you can quickly see the amount of time between events. For example, you could see that, at 12 seconds after adding an agonist solution, a two-fold increase of cytoplasmic calcium was detectable. It is often not necessary or desirable to relate these events to the actual time of day.

Converting the Clock's Time

If you need to know the actual time of day that an event occurred, you can convert the clock's seconds. For example, if you zeroed the clock at 3:30 PM, and a data point registered on the graph at 1372 seconds, you could divide 1372 by 60 seconds / minute to see that 1372 is 22 minutes and 52 seconds. Then simply add this to your start time of 3:30:00 PM to find that the event actually occurred at 3:52:52 PM.

Shortcut: CTRL + Z

See Also:

Clear Graphs

Zeroing the Clock

To zero the clock, use the following procedure:

Step	Action
1	Select the Run Experiment menu.
2	Choose Zero Clock. The clock for data logging and graphing will be reset to 0.0.

Note: You can use CTRL + Z, the keyboard shortcut for the Zero Clock command, to reset the clock at any point in the experiment.

Set Timelapse

Sets the timelapse interval for acquiring images for a defined number of acquisitions.

Use this option to acquire images at equally-spaced intervals, limited by the number of acquisitions that you specified. If you specify Zero as the number of acquisitions, Metafluor will acquire images indefinitely. You can specify the unit of measure for the time interval as milliseconds, seconds, minutes, or hours.

Setting Timelapse Intervals

To set timelapse, complete the following procedure.

Step	Action
1	From the Experiment Control Panel, click *Set Timelapse* The Set Timelapse dialog box opens.
2	Type or select the interval length and select the interval unit of measure. (milliseconds, seconds, minutes, or hours).
3	Type or select the number of acquisitions. Set this value to zero to acquire images indefinitely.
4	Click closed to close the dialog box.

Set Timelapse - Dialog Box Options

Timelapse Interval

The amount of time between acquisitions in milliseconds, seconds, minutes, or hours.

Number of Acquisitions

The total number of images that you want to acquire for your experiment. To acquire images indefinitely, set this value to Zero (0).

Close

Closes the *Set Timelapse* dialog box.

Save Last Acquired Images (Run Experiment Menu)

Saves the last set of acquired images to disk.

Use this command to save acquired images to disk. If you have an .inf file already open, the last set of acquired images will be saved using the next sequence number (002, etc.) and the .inf file will be updated.

Shortcut: [F7]

Saving the Last Acquired Images

To save the last acquired images, use the following procedure:

Step	Action
1	Open an .inf file for saving wavelength images. (This can be done by selecting *Save Images* in the Experiment Control Panel.)
2	Acquire at least one set of images using the Experiment Control Panel.
3	From the Run Experiment menu, choose Save Last Acquired Images. The last set of wavelength images will be saved.
	Note: If you want to save the last set of images during acquisition, you can use the keyboard shortcut by pressing the [F7] key at any time, rather than following Step 3.

Log Now (F9) (Run Experiment Menu)

Logs the current region data to an open measurements file.

Use this command to log region data (as defined by the current regions and the status of Configure Experiment) to an open measurements file. A typical use would be when you want to log the quantitative data from particular frames in a stored experiment that you are playing back. You do **not** need to select *Log Data* in the Experiment Control Panel, but you **do** need to open a measurements file before you use this command.

Shortcut: [F9]

See Also:

Open Measurements File

Configure Experiment

Experiment Control Panel - Playback Mode

Using Log Now

To log region data from an image to a measurements file, use the following procedure:

Step	Action
1	Open a measurements file using the **Open Measurements File** command.
2	Where appropriate, use the **Configure Experiment** command to select the data to be logged.
3	From the Run Experiment menu, choose Log Now. **OR** Press [F9] to log the data using the keyboard shortcut.
4	The measured data will be logged.

Scale 16-Bit Images (Run Experiment Menu)

Scales the gray level display for 16-bit images to an 8-bit (256-level) range of gray levels of your choice, thereby increasing the image contrast without affecting the data.

This command allows you to scale 16-bit images to a selected range of 256 gray levels. (MetaFluor considers any image that has more than 8 bits per pixel to be a 16-bit image, even if the image is actually only 10 or 12 bits.) Due to experimental conditions, much of the important image data may reside within a narrow range of gray levels, and displaying the full 16-bit range may make it difficult or impossible to see intensity differences. Scaling a selected range will allow you to see those differences. The **scaling process** expands the range of gray levels in the 16-bit image to an 8-bit display.

Scaling a 16-Bit Image
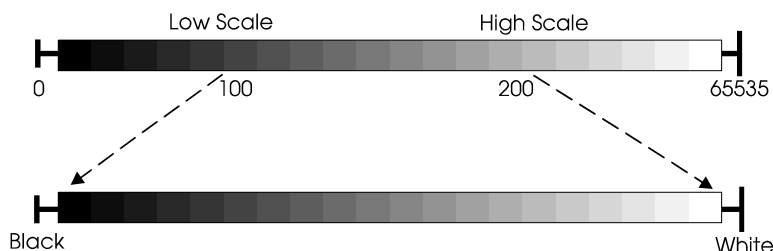
Note: This command does not affect pixel intensity values; its only purpose is to give you the option of selecting a range of gray levels to display.

The scaling process requires a minimum and a maximum gray level (either determined during autoscaling or specified by you). When scaling, MetaFluor takes the range of gray levels in the 16-bit image that fall between the minimum and the maximum gray level, and divides them by 256. Each of these resulting bins is displayed at the same gray level on the monitor. If your image contains outlying "hot" pixels (either oversaturated or undersaturated), you can exclude a selected percentage of the pixels in the image (not the gray levels in its histogram!) at the lower and upper end of the image's grayscale range by adjusting the settings of the *Lo%* and *Hi%* spin boxes, respectively. For example, you could exclude the bright nucleus of a cell in a fluorescence image by setting the low spin box to 1% and the high spin box to 10%. (You can specify a fractional value, such as 0.1%, if necessary. **Click here** for a depiction of a sample histogram with the lowest and highest 0.1% of pixels selected for exclusion.) Scaling will then be based on the lowest and highest remaining grayscale values.

You can

(1) **Autoscale** all wavelengths,

(2) Set the same fixed scaling for all wavelengths,

(3) Set a different fixed scaling for each of the wavelengths, or

(4) Use any combination of autoscaled and fixed scalings for each of the wavelengths.

In addition, you can specify that the scaling for Wavelength 1 and 2, or that for Wavelength 4 and 5, are to be the same. This will simplify pairing of images for ratiometric measurement.

Note: If you set the grayscale histogram scaling and axis range for an image with its Histogram Tool, this will update the settings for the selected wavelength in the Scale 16-Bit Images dialog box (as long as autoscaling has been disabled).

Scaling 16-Bit Images

To scale 16-bit images, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Scale 16-Bit Images. The Scale 16-Bit Images dialog box will appear.
2	If you want MetaFluor to scale all images automatically based on their lowest and highest grayscale values, choose *Autoscale All.* Then skip to Step 6.
3	Select *Wavelength 1* from the *Select a Wavelength to Adjust Its Scaling* table.
4	If you want MetaFluor to scale the image automatically based on its lowest and highest grayscale values, select the *Autoscale* check box. If desired, specify a percentage of pixels (not gray values) to be excluded from the lower and upper ends of the scaling ranges with the *Lo%* and *Hi%* spin boxes, respectively.
	OR Clear the *Autoscale* check box. Then select the lowest and highest grayscale values to be represented in the image using the *Low* and *High* spin boxes, respectively, or drag the lower and upper indicators to the desired positions along the scale slider.
5	To use the same scaling for Wavelength 2 that you just selected for Wavelength 1, choose *Set W2 = W1.*
	OR If you want all of the remaining wavelength images to use the same scaling as the one you selected in Step 4, choose *Set All Equal.*
	OR If you want to have a different scaling for the remaining wavelength images, repeat Steps 3 and 4 for the other images. Where appropriate, you can use the same scaling for Wavelength 5 that you select for Wavelength 4, by choosing *Set W5 = W4.*
6	When you have finished, choose *Close.*

Scale 16-Bit Images - Dialog Box Options

Select a Wavelength to Adjust Its Scaling

Selects the image that you want to configure.

Low

Specifies the darkest gray level for the image(s). When you select the *Autoscale* check box, this option becomes the *Lo%* spin box.

High

Specifies the brightest gray level for the image(s). When you select the *Autoscale* check box, this option becomes the *Hi%* spin box.

Autoscale

Configures the selected image to be **autoscaled.**

Lo%

Excludes a selected percentage of pixels in the image (not the percentage of gray levels in the histogram) from the low (darker) end of the range of values being autoscaled. This option is available only when the *Autoscale* check box has been selected.

Hi%

Excludes a selected percentage of pixels in the image (not the percentage of gray levels in the histogram) from the high (brighter) end of the range of values being autoscaled. This option is available only when the *Autoscale* check box has been selected.

Scale Slider

Selects a lower and upper gray value for the scaling range. Drag the indicators to the desired positions along the slider to select the grayscale values.

Set W2 = W1

Configures Wavelengths 1 and 2 to use the same fixed scaling. If, for example, *Wavelength 1* is currently selected in the *Select a Wavelength…* table, this option will set the scaling for Wavelength 2 to that currently selected for Wavelength 1. If you select *Wavelength 2* from the *Select a Wavelength…* table, the label on this command button will change to *"Set W1 = W2,"* and choosing the button will configure Wavelength 1 to use the setting for Wavelength 2.

Set W5 = W4

Configures Wavelengths 4 and 5 to use the same fixed scaling. If, for example, *Wavelength 4* is currently selected in the *Select a Wavelength…* table, this option will set the scaling for Wavelength 5 to that currently selected for Wavelength 4. If you select *Wavelength 5* from the *Select a Wavelength…* table, the label on this command button will change to *"Set W4 = W5,"* and choosing the button will configure Wavelength 4 to use the setting for Wavelength 5.

Set All Equal

Configures all images to use the same fixed scaling. If you select the *Autoscale* check box, and then choose *Set All Wavelengths to the Same Scaling,* this will have the same effect as choosing *Autoscale All Wavelengths.*

Autoscale All

Configures all images to be **autoscaled.**

Close

Closes the dialog box.

Selecting a Video Device

Selecting a Video Device

To select a video device, use the following procedure:

Step	Action
1	From the File menu, choose Select Video/Camera for Acquisition. The Select Video/Camera for Acquisition dialog box will appear.
2	From the *Video Driver* drop-down list, select the video device you want to use.
3	From the *Video Channel* drop-down list, select the video channel to be used. The options you see will depend on your hardware configuration.
4	Choose *OK.*

Select Video/Camera for Acquisition - Dialog Box Options

Video Driver

Allows you to temporarily select a different video device from among those you currently have installed in the Video Driver Manager.

Video Channel

Selects the video channel to be used. The options you see will depend on your hardware configuration.

OK

Changes to the selected video device.

Cancel

Cancels the command.

Video Cameras

Experiment Control Panel (Run Experiment Menu) - Video Camera and External Monitor

Contains the live video, reference image, and image and data acquisition commands needed to run a new experiment using an analog video camera, a video acquisition board.

The Experiment Control Panel dialog box is your "command center" for controlling new experiments. Using it, you can adjust the camera's analog contrast and focus the microscope, acquire reference images, specify image acquisition settings, and enable image saving and data logging.

You can use the *Focus* command button to obtain continuous images while focusing the microscope prior to starting the experiment. The Focus Video Using External Monitor dialog box also contains controls for adjusting the analog contrast (white and black levels) while monitoring for under- and oversaturation. As you focus the microscope, MetaFluor continuously acquires and displays images which you can use to verify that your specimen is visible and in focus. It is important to use the *Focus* command while you are focusing the microscope, because what can be seen through the microscope's eyepiece is not always the same as what the camera acquires.

Most of the options in the Experiment Control Panel are image and data acquisition controls. These are used to enable image and data saving prior to starting your experiment. "LED" indicators next to the *Save Images* and *Save Ratios* check boxes will indicate the saving status of each Wavelength and Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Wavelength or Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle. When you have selected all of the necessary options, the experiment can be run. You can enable background subtraction and shading correction using the **Reference Images** command.

Before you start acquisition, spend a minute to make sure that **Image Display Controls** and **Configure Experiment** are set appropriately for your experiment. You may also want to check the Data Logging Preferences options (Preferences command, File menu) if you are logging data. If you plan to use event marks or move regions while running the experiment, you may want to open and position those dialog boxes before starting acquisition.

Note: This version of the Experiment Control Panel is unavailable in the MetaFluor Offline system. Please see the description of the **Playback Mode version.**

See Also:

Experiment Control Panel:

For Video Camera with Computer's Monitor

For Playback

Image Display Controls - Using an External Video Monitor

Configure Experiment

Preferences

Reference Images

Adjusting the Image Using a Video Camera with an External Video Monitor

To adjust the image while displaying live video on the external monitor, use the following procedure.

Note: You can make separate analog adjustments for each video channel.

Step	Action
1	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel will appear. The *Status* line will indicate that you are using Live Video.
2	Choose *Focus.* The Focus Video Using External Monitor dialog box will appear.
3	If an open shutter is needed, select the *Open Shutter* check box, located on the left below the *Wavelength* table.
4	Select *Wavelength 1* from the *Wavelength* list. This will display live video for Wavelength 1 on the external video monitor. You should use this image to focus the microscope.
5	Select *Use Saturation Warning Markers* to display warning markers on the video monitor to indicate areas of undersaturation (too dark--will be clipped to 0) and oversaturation (too bright--will be clipped to maximum possible value [255 for 8-bit images]). MetaFluor uses the following colors for markers:

Dark Blue	Undersaturated--too dark
Cyan	Approaching undersaturation
Yellow	Approaching oversaturation
Red	Oversaturated--too bright.

Step	Action
6	The Scan Line Plot graphs the grayscale values under a red horizontal scan line placed over the live video image. To move the scan line so that it displays the desired areas of undersaturation or oversaturation, move the vertical slider located to the left of the Scan Line Plot.
7	Use the *White Level* and *Black Level* sliders at the top of the dialog box to adjust the contrast so that the scan line covers most of the available grayscale values (displayed on the graph's X-axis), without introducing the Saturation Warning Markers on the video monitor.
8	If you opened a shutter in Step 3, close it now by clearing the *Open Shutter* check box.
9	If you want all wavelengths to use the same settings, select the *All Wavelengths Use Same Analog Settings* check box. Then skip to Step

11.

OR
If you want to adjust the settings separately for each wavelength, continue to Step 10.

10   After you have adjusted the *White Level* and *Black Level* so that they are suitable for Wavelength 1, select *Wavelength 2* from the *Wavelength* table. Then repeat Steps 3 - 8 for Wavelength 2. You will probably need to toggle between the two wavelengths to make sure the analog adjustments are appropriate for both. Repeat as necessary for the remaining wavelength images.

11   Choose *Close* to return to the Experiment Control Panel.

Experiment Control Panel (Run Experiment Menu) - Video Camera with Computer's Monitor

Contains the focusing and image and data acquisition commands needed to run a new experiment using a video camera while displaying your data images in image windows on your computer screen.

The Experiment Control Panel is your "command center" for controlling new experiments. Using it, you can focus the camera, set image acquisition settings, and enable image saving and data logging. If your camera has gain and offset controls, you can adjust these while focusing the camera.

You can use the *Focus* command button to obtain continuous images while focusing the microscope prior to starting the experiment. As you focus the microscope, MetaFluor continuously acquires and displays images which you can use to verify that your specimen is visible and in focus. It is important to use the *Focus* command while you are focusing the microscope, because what can be seen through the microscope's eyepiece is not always the same as what the camera acquires. The *Focus* command will summon the Focus dialog box, which you can use to select a wavelength image to use for focusing. You can also adjust the analog contrast of the image from this dialog box. Although you are displaying your acquired data images in image windows on the computer monitor, you can display your focusing image in either an image window on the computer monitor or you can display it on an external monitor. This will be determined by whether or not you select the *Check If an External Monitor Is Attached…* check box in the Video Camera Preferences dialog box (Preferences command, File menu). The Focus dialog box that you see will depend on whether you are using an external video monitor to view the focusing image.

The majority of the commands in the Experiment Control Panel are image and data acquisition commands. Most are used prior to starting an experiment to define the acquisition. When you have selected all of the necessary options, the experiment can be run. "LED" indicators next to the *Save Images* and *Save Ratios* check boxes will indicate the saving status of each Wavelength and Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Wavelength or Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle. You can enable background subtraction and shading correction using the **Reference Images** command.

Before you start acquisition, spend a minute to make sure that **Image Display Controls** and **Configure Experiment** are set appropriately for your experiment. You may also want to check the Data Logging Preferences options (Preferences command, File menu) if you are logging data. If you plan to use event marks or move regions while running the experiment, you may want to open and position those dialog boxes before starting acquisition.

Note: This version of the Experiment Control Panel is unavailable in the MetaFluor Offline system. Please see the description of the **Playback Mode version.**

See Also:

Experiment Control Panel:

For Video Camera and External Monitor

For Video Camera with Other Video Board and External Monitor

For Playback

Reference Images

Image Display Controls - Using the Computer's Monitor

Preferences

Event Marks

Experiment Control Panel - Dialog Box Options (Video Camera with Computer's Monitor)

Status

Reports the length of time to the next acquisition, as set by *Set Timelapse* and the shutter delay time in the Illumination Control dialog box (the *Delay* option). The message "Running" occurs when an image is acquired or when the requested acquisition interval is shorter than the time required to complete the acquisition cycle.

Log Data

Enables or disables data logging to an open, DDE-linked spreadsheet file and/or text file. The status line next to *Log Data* will indicate if a file is open for saving data. If a data file has not been opened prior to selecting *Log Data,* the Open Measurements File command will be activated automatically so that you can open a file.

Save Images

Enables or disables wavelength image saving. The status line next to *Save Images* will indicate if an .inf file is open. If an .inf file has not been opened prior to selecting *Save Images,* the Open Save Images File command will be activated automatically so that you can open an .inf file. "LED" indicators next to the *Save Images* check box will indicate the saving status of each Wavelength image: gray indicates that saving is off, red indicates that saving is on but that the particular Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle.

Save Ratios

Enables or disables ratio image saving. The status line next to *Save Ratios* will indicate when you can save ratio images. "LED" indicators next to the *Save Ratios* check box will indicate the saving status of each Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle. The Open Ratios File command will be activated automatically when you select *Save Ratios.*

F4: Acquire

Acquires image pairs and ratios them according the display and acquisition options. When this command is chosen, the text on its button will change to *"F2: Pause."*

F2: Pause

Pauses acquisition at the end of the present cycle. You can also press the [F2] key on the keyboard to stop acquisition.

Set Timelapse

Sets the time between acquisitions and the number of acquisition to be completed. Select *0* for the *Timelapse Interval* if you do not want to use timelapsing. Select *0* for the *Number of Acquisitions* if you want acquisition to continue until you choose *Pause Acquisition.*

Zero Clock

Same as the Zero Clock command in the Run Experiment menu.

Focus

Opens the Focus dialog box. The dialog box that you see will depend on whether you are using an external video monitor to view the focusing image. This will in turn be determined by whether or not you select the *Check If an External Monitor Is Attached…* check box in the Video Camera Preferences dialog box (Preferences command, File menu).

Cycle and CPS

One cycle is the total time required to acquire both wavelength images (with background subtraction and/or shading correction), display them, ratio them, display the ratio image, draw regions (if selected), make measurements, plot measurements on graphs, log data, and save images. The CPS calculation is updated every 10 uninterrupted cycles.

Close

Closes the dialog box.

Focusing the Video Camera Using the Computer Monitor

To focus the image when you are using the computer monitor to display both the focusing and data images, use the following procedure:

Step	Action
1	From the Experiment Control Panel, choose *Focus.* The Focus dialog box will appear. MetaFluor will temporarily close all other dialog boxes that are open.
2	From the *Wavelength* table on the right side of the dialog box, select the wavelength image you want to use for focusing.
3	From the *Frames to Average* spin box, select the number of frames to average together in the focusing image. Select *1* for no averaging. (If you change the settings from those you configured using the Configure Acquisition command, you can direct MetaFluor to use the new settings for acquiring the data images by choosing *Store for Acquisition.)*
	OR
	If you want to use the same settings for averaging as you configured for the data images with the Configure Acquisition command, choose *Use Wave Settings.*
4	If you are using an external shutter, select *Open on Start Focus.* Select *Close After Acquire* if you want the shutter to close immediately after acquiring a focusing image.
5	When you are ready to start acquiring focus images, choose the *Start Focusing* button. If you are using a shutter, choose *Toggle Shutter* to open the shutter. Choose it again to close the shutter. The colored box to the right of the button will indicate when the shutter is open or closed. After you choose *Start Focusing,* a new *Interactive* option group will appear below the *Acquisition Region* option group (see Step 6, middle option).
	You can pause acquisition at any time by choosing *F2: Pause Focus* or by pressing the [F2] function key. Resume focusing by choosing *F4: Resume Focus* or by pressing the [F4] function key.
6	If you want to use a subregion of the image for focusing, use the box-in-box display or the *Left, Top, Width,* and *Height* spin boxes to specify the size and location of the focusing subregion. Choose *CTR* to place the center of the region in the center of the image. Alternatively, choose *Full Chip* to use the

entire image. If you want to use this newly configured region for acquisition as well, choose *Store as Acq Rgn.*

OR

Choose the *Select* button in the new *Interactive* option group that appears in the upper left area of the Focus dialog box. This will display a green region of interest on the Focus image window. Resize and reposition the region as needed with your cursor and choose *OK* to fill the entire Focus image window with the selected region.

OR

If you want to use the same acquisition region settings as you configured for the data image itself with the Configure Acquisition command, choose *Use Acq. Region.*

7   While monitoring the focusing image, adjust the microscope to provide the optimum focus.

8   If you want to adjust the contrast in the image, use the *White Level* and *Black Level* sliders to adjust the camera's white level and black level settings.

Select *Use Saturation Warning Markers* to display warning markers on the focusing image to indicate areas of undersaturation (too dark--will be clipped to 0) and oversaturation (too bright--will be clipped to maximum possible value [255 for 8-bit images]). MetaFluor uses the following colors for markers:

Dark Blue   Undersaturated--too dark
Cyan   Approaching undersaturation
Yellow   Approaching oversaturation
Red   Oversaturated--too bright.

9   If you want to view a graphical display of the intensity values in the image while adjusting the contrast, select the *Update* check box, select either *Histogram* or *Scanline* from the drop-down list, and choose the image's bit-depth from the *Depth* list.

The histogram or line graph can be configured as needed from the Down Arrow configuration menu.

10   If you want all wavelengths to use the same contrast settings, select the *All Wavelengths Use Same Analog Settings* check box. Then skip to Step 12.

OR

If you want to adjust the settings separately for each wavelength, continue to Step 11.

11   After you have adjusted the *White Level* and *Black Level* so that they are suitable for Wavelength 1, select *Wavelength 2* from the *Wavelength* table. Then repeat Steps 3 - 9 for Wavelength 2. You will probably need to

toggle between the two wavelengths to make sure the analog adjustments are appropriate for both. Repeat as necessary for the remaining wavelength images.

12 When you have finished, toggle the shutter closed (if necessary) and choose *Stop Focusing.*

13 Choose *Close* to return to the Experiment Control Panel.

Focus - Dialog Box Options (Computer Monitor Display of Focus Image from Video Camera with No External Monitor)

Binning

Configures pixel binning for acquisition of focusing images from a digital camera. This option will be unavailable.

Left

Defines and displays the leftmost point of the region.

Top

Defines and displays the topmost point of the region.

Width

Defines and displays the width of the region.

Height

Defines and displays the height of the region.

Use Acq Region

Directs MetaFluor to use the acquisition region settings from the Configure Acquisition dialog box for the focusing image.

Store as Acq Rgn

Directs MetaFluor to use the current Focus dialog box focusing acquisition region for acquisition of the data images. These settings will be stored in the Configure Acquisition dialog box.

Select (Interactive)

Displays a green region of interest on the Focus image window. After you reposition and resize the region around the area in the image that is of greatest importance, choosing *OK* will zoom that area to fill the entire Focus image window. A green "LED" will flash beneath the *OK* and *Cancel* buttons while the *Select* function is active. The *Interactive* option group *(Select, OK,* and *Cancel)* appears when you choose *Start Focusing.*

OK (Interactive)

Accepts the green region of interest drawn in the Focus image window when you choose the *Select* button, and zooms the selected area of the image to fill the entire Focus image window. The *Interactive* option group *(Select, OK,* and *Cancel)* appears when you choose *Start Focusing.*

Cancel (Interactive)

Cancels the selection of the image area selected by the green region of interest, and removes the region from the Focus image window.

<<

Decreases the size of the focusing image's acquisition subregion by half.

CTR

Centers the focusing image acquisition subregion.

>>

Doubles the size of the focusing image's acquisition subregion.

Full Chip

Specifies that the entire camera chip be used as the focusing image's acquisition subregion.

Box-in-Box Display

Allows you to define a region of interest for focusing. Drag the outline of the smaller box to resize and position it, just as you would for a data acquisition subregion.

Frames to Average

Selects the number of frames to be averaged for each focusing image.

Use Wave Settings

Directs MetaFluor to use the frame averaging settings from the Configure Acquisition dialog box for the focusing image.

Store for Acquisition

Directs MetaFluor to use the current Focus dialog box camera settings for acquisition of the data images. These settings will be stored in the Configure Acquisition dialog box.

Update

Enables continuous updating of the intensity values in the histogram or line graph while you configure contrast.

Histogram/Scanline Selection List

Selects a display mode for the continously updating intensity graph: *Histogram* or *Scanline.* If you select Histogram, the gray values in the entire image (or focus region) will be numerically represented in the histogram bins. If you select *Scanline,* you can select the location of the red scanline in the image by dragging it up or down in the image window, and the gray values under the line will be represented in a line graph.

Depth

Selects a range of intensity values for display in the intensity graph. The value should reflect the bit-depth of the camera. Select *8-Bit* for a range of 0 - 256, *10-Bit* for a range of 0 - 1024, or *12-Bit* for a range of 0 - 4096.

Intensity Graph

Displays the continuously updated intensity values in the focusing image in either a histogram or line graph.

Down Arrow Configuration Menu

Allows you to configure the intensity graph. You can also print the graph or copy it to the Clipboard. (For more information, see **Graphs).**

Wavelength

Selects a wavelength image for display of the focusing image.

Toggle Shutter

Toggles the shutter open and closed. The colored box to the right of the button will indicate when the shutter is open or closed.

Open on Start Focus

Directs MetaFluor to open the shutter at the start of the focus image's acquisition cycle.

Close After Acquire

Directs MetaFluor to close the shutter at the end of the focus image's acquisition cycle. Leave this check box cleared to perform continuous acquisition.

White Level

Adjusts the white level (signal voltage representing the brightest gray level in the image) of the focusing image.

Black Level

Adjusts the black level (signal voltage representing the darkest gray level in the image) of the focusing image.

Use Saturation Markers

Displays saturation markers on the video monitor and the video image, indicating areas of undersaturation (dark blue--too dark, will be clipped to zero) and oversaturation (red--too bright, will be clipped to 255).

All Wavelengths Use Same Analog Settings

When selected, this check box directs MetaFluor to use the same white level and black level settings for all wavelengths. If you clear this check box, you can configure the settings separately for each wavelength that you select from the *Wavelength* table. The default state for the check box is to be selected.

Start Focusing / Stop Focusing

Starts and stops the acquisition of images for focusing.

F2: Pause Focus

Pauses acquisition of the focusing images.

F4: Resume Focus

Resumes acquisition of the focusing images.

Close

Closes the dialog box.

Experiment Control Panel - Dialog Box Options (Video Camera with External Monitor)

Status

Reports the length of time to the next acquisition, as set by *Set Timelapse* and the shutter delay time in the Illumination Control dialog box (the *Delay* option). The message "Running" occurs when an image is acquired or when the requested acquisition interval is shorter than the time required to complete the acquisition cycle.

Log Data

Enables or disables data logging to an open, DDE-linked spreadsheet file and/or text file. The status line next to *Log Data* will indicate if a file is open for saving data. If a data file has not been opened prior to selecting *Log Data,* the Open Measurements File command will be activated automatically so that you can open a file.

Save Images

Enables or disables wavelength image saving. The status line next to *Save Images* will indicate if an .inf file is open. If an .inf file has not been opened prior to selecting *Save Images,* the Open Save Images File command will be activated automatically so that you can open an .inf file. "LED" indicators next to the *Save Images* check box will indicate the saving status of each Wavelength image: gray indicates that saving is off, red indicates that saving is on but that the particular Wavelength image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle.

Save Ratios

Enables or disables ratio image saving. The status line next to *Save Ratios* will indicate when you can save ratio images. "LED" indicators next to the *Save Ratios* check box will indicate the saving status of each Ratio image: gray indicates that saving is off, red indicates that saving is on but that the particular Ratio image will not be saved, blue indicates that saving is on and that the image will be saved, but not on every cycle, and green indicates that the image will be saved on every cycle. The Open Ratios File command will be activated automatically when you select *Save Ratios.*

F4: Acquire

Acquires wavelength image and ratios them according the display and acquisition options. When you choose this command, its label will change to *"F2: Pause."*

Set Timelapse

Sets the time between acquisitions and the number of acquisition to be completed. Select *0* for the *Timelapse Interval* if you do not want to use timelapsing. Select *0* for the *Number of Acquisitions* if you want acquisition to continue until you choose *F2: Pause.*

F2: Pause

Pauses acquisition at the end of the present cycle. You can also press the [F2] key on the keyboard to stop acquisition.

Zero Clock

Same as the Zero Clock command in the Run Experiment menu.

Focus

Opens the **Focus Video Using External Monitor** dialog box, which you can use to adjust the white and black levels and to control acquisition while you focus the microscope.

Cycle and CPS

One cycle is the total time required to acquire both wavelength images (with background subtraction and/or shading correction), display them, ratio them, display the ratio image, draw regions (if selected), make measurements, plot measurements on graphs, log data, and save images. The CPS calculation is updated every 10 uninterrupted cycles.

Close

Closes the dialog box.

Analog Contrast - Dialog Box Options

White Level

Adjusts the white level (signal voltage representing the brightest gray level in the image) of the incoming image on the video monitor.

Black Level

Adjusts the black level (signal voltage representing the darkest gray level in the image) of the incoming image on the video monitor.

Use Saturation Markers

Displays saturation markers on the video monitor and the video image, indicating areas of undersaturation (dark blue--too dark, will be clipped to zero) and oversaturation (red--too bright, will be clipped to 255).

More >>

Expands the dialog box to display the scan line plot.

Less <<

Condenses the dialog box.

Scan Line Plot

Graphs the grayscale values under the horizontal scan line that is placed over the video image window. The vertical slider located to the left of the scan line plot determines the areas of undersaturation or oversaturation that have been plotted.

Show Scan Line

Selecting this check box displays the scan line directly on the external video monitor. **Note:** Doing so may slow down your video display.

Down Arrow Configuration Menu

Allows you to configure the scan line plot graph. You can also print the graph or copy it to the Clipboard. (For more information, see **Graphs).**

Close

Closes the dialog box.

Focusing the Video Camera Using an External Video Monitor

To focus the image using an external video monitor when you are displaying your data images on the computer monitor, use the following procedure:

Step	Action
1	From the Experiment Control Panel, choose *Focus.* The Focus dialog box will appear. MetaFluor will temporarily close all other dialog boxes that are open.
2	From the *Switch To* radio button group, select which wavelength image you want to use for focusing.
3	When you are ready to begin focusing, select the *Open Shutter* check box if you have an external shutter attached to the camera.
4	Focus your microscope.
5	If you want to adjust the contrast in the image, continue to Step 6. **OR** If you do not need to adjust the image contrast, skip to Step 11.
6	Choose *Adjust Analog.* The Analog Contrast dialog box will appear.
7	Select *Use Saturation Warning Markers* to display warning markers on the video monitor to indicate areas of undersaturation (too dark--will be clipped to 0) and oversaturation (too bright--will be clipped to maximum possible value [255 for 8-bit images]). MetaFluor uses the following colors for markers:

Dark Blue   Undersaturated--too dark
Cyan   Approaching undersaturation
Yellow   Approaching oversaturation
Red   Oversaturated--too bright.

8	The *Scan Line Plot* graphs the grayscale values under a red horizontal scan line placed over the live video image. To display the scan line on your video monitor, select the *Show Scan Bar* check box so that a check mark appears in the box. **AND** To move the scan line so that it displays the desired areas of undersaturation or oversaturation, move the vertical slider located to the left of the *Scan Line Plot.*
9	Use the *White Level* and *Black Level* sliders at the top of the dialog box to adjust the contrast so that the scan line covers most of the available grayscale values (displayed on the

graph's X-axis), without introducing the Saturation Warning Markers on the video monitor.

10   After you have adjusted the *White Level* and *Black Level* so that they are suitable for the selected wavelength image, select a different wavelength image from the Experiment Control Panel's *Switch To* group. Then repeat Steps 6 - 9 for that image. You will probably need to toggle between the two wavelengths to make sure the analog adjustments are appropriate for both.

Repeat as necessary for any other wavelengths, selecting them from the *Switch To* group and repeating Steps 6 - 9.

11   When you have finished, clear the *Open Shutter* check box. Then choose *Close* to return to the Experiment Control Panel.

Focus Video Using External Monitor - Dialog Box Options

Wavelength

Selects a wavelength image, so that you can adjust the analog contrast for each wavelength and focus the microscope while in Live Video mode.

Open Shutter

Opens the shutter. If you have the acquisition wavelengths configured to use different MetaDevices, you will be able to use separate *Open Shutter* settings for each wavelength. This allows you to operate two shutters, one from each MetaDevice.

All Wavelengths Use Same Analog Settings

When selected, this check box directs MetaFluor to use the same white level and black level settings for all wavelengths. If you clear this check box, you can configure the settings separately for each wavelength that you select from the *Wavelength* table. The default state for the check box is to be selected.

White Level

Adjusts the white level (signal voltage representing the brightest gray level in the image) of the incoming image on the video monitor.

Black Level

Adjusts the black level (signal voltage representing the darkest gray level in the image) of the incoming image on the video monitor.

Use Saturation Markers

Displays saturation markers on the video monitor and the video image, indicating areas of undersaturation (dark blue--too dark, will be clipped to zero) and oversaturation (red--too bright, will be clipped to 255).

Show Graph >>

Expands the dialog box to display the scan line plot.

Hide Graph <<

Condenses the dialog box.

Scan Line Plot

Graphs the grayscale values under the horizontal scan line that is placed over the video image window. The vertical slider located to the left of the scan line plot determines the areas of undersaturation or oversaturation that have been plotted.

Down Arrow Configuration Menu

Allows you to configure the scan line plot graph. You can also print the graph or copy it to the Clipboard. (For more information, see **Graphs).**

Close

Closes the dialog box.

Focus - Dialog Box Options (Computer Monitor Display of Focus Image from Video Camera with External Monitor Present)

Switch To

Selects a wavelength image for focusing or adjusting contrast.

Open Shutter

Toggles the shutter open and closed.

Adjust Analog

Opens the **Analog Contrast** dialog box.

Close

Closes the dialog box.

Image Display Controls - Dialog Box Options (Using an External Video Monitor)

Image

Selects the wavelength image that you want to threshold. This option affects only the threshold settings.

Low

Use this text box or the left handle of the slider to specify the lowest gray value in the selected image.

High

Use this text box or the right handle of the slider to specify the highest gray value in the selected image.

Image Display Mode

Specifies the display mode for the wavelength images as either *Monochrome Images* or *Pseudocolor Images.*

Minimum

Selects the minimum ratio value for the ratio image.

Maximum

Selects the maximum ratio value for the ratio image.

Ratio Display

Selects the desired IMD display for the ratio image. The IMD display will use a custom look-up table that consists of hues corresponding to the selected number of ratios, each having the specified number of intensities. For example, a ratio image that was built using *8 Ratios with 32 Intensities* will have 8 distinct hues, each with 32 intensities, visible in its contrast/threshold slider (as opposed to the continuous range of values visible for a pseudocolor image).

IMD Intensity

Selects the source for the intensity values. Select the brighter wavelength image. If you don't know which image will be brighter, select *Average Num. and Denom.* instead.

Apply

Applies the image display control settings. The settings will take effect the next time you acquire images.

Close

Closes the dialog box.

Image Display Controls - Dialog Box Options (Using the Computer's Monitor)

Window

Selects the wavelength or ratio image for which you want to change the image display settings.

Display

Specifies the display mode for the wavelength images as either *Monochrome* or *Pseudocolor*. Specifies the display mode for the ratio image as *Monochrome, Pseudocolor,* or *IMD Display,* and displays the appropriate options for other image display settings based on this selection.

Brightness

Allows you to adjust the overall brightness of an image. The default value is *50.* Minimum brightness is *0;* maximum brightness is *100.*

Contrast

Allows you to expand the range of grayscale levels displayed for an image. The default value is *50.* Increasing the value raises the contrast. Maximum contrast *(100)* produces a binary image. Contrast can not be decreased.

Low Thresh

Defines the lowest gray value in the selected wavelength image.

High Thresh

Defines highest lowest gray value in the selected wavelength image.

IMD Display

If you have selected *IMD Display* as the *Display* and are displaying ratio images as 8-bit images (Preferences : General command, File menu), use this option to select the desired IMD display for the ratio image using this option. The IMD display will use a custom look-up table that consists of hues that correspond to the selected number of ratios, each having the specified number of intensities. For example, a ratio image that was built using *8 Ratios with 32 Intensities* will have eight distinct hues, each with 32 intensities, visible in its contrast/threshold slider (as opposed to the continuous range of values visible for a pseudocolor image).

IMD Intensity

Selects the source for the intensity values in the ratio image. Select the brighter wavelength image. If you don't know which image will be brighter and you have selected an 8-bit ratio display (Preferences : General command, File menu), select *Average Num. and Denom*. instead. If you have selected a 24-bit ratio display (Preferences: General command, File menu), you will also have the option of selecting either the mean intensity *(Average of Wavelengths)* or the maximum intensity *(Maximum of Wavelengths)* of all wavelength images as the intensity component. The *IMD Intensity* option will appear only when you select *IMD Display* from the *Display* drop-down list.

IMD Overlay

Selects the source for the saturation component of 24-bit ratio images. You can select any of the wavelength images. If you select *None,* the maximum saturation will be used. The *IMD Overlay* option will appear only when you select *IMD Display* from the *Display* drop-down list and configure MetaFluor to use a 24-bit ratio image display (Preferences : General command, File menu).

Min. Ratio

Selects the minimum ratio value for the ratio image.

Max. Ratio

Selects the maximum ratio value for the ratio image.

Close

Accepts the current settings and closes the dialog box.

Reference Images

Reference Images - External Monitor Display (Run Experiment Menu)

Saves or loads background reference images and white (shading) reference images for correction of images being acquired or played back and displayed on an external video monitor. Can also create artificial background images using specified gray levels.

Use this command to save background and/or white (shading) reference images that you have acquired using the Experiment Control Panel. After you have saved these images, you can use the Reference Images command to load them later for background subtraction and shading correction. You can also use this command to create artificial background images, each with their own specified grayscale value. You may want to do so to subtract an offset from each wavelength.

Note: You will need to use a different **Reference Images** dialog box when displaying images in image windows on the computer desktop.

See Also:

Experiment Control Panel

Reference Images - Window Display

Saving Reference Images (External Monitor Display)

To save a set of reference images, use the following procedure:

Step	Action
1	Acquire the desired reference images using either *Acq. Background* or *Acquire Shading* in the Experiment Control Panel.
2	From the Run Experiment menu, choose Reference Images. The Reference Images dialog box will appear.
3	If you want to save a background image, choose the *Save to Disk* button below the *Background References* label. The *Save Backgrounds* dialog box will appear. **OR** Choose *Save to Disk* below the *White References* label. The *Save White Reference* dialog box will appear.
4	Type a new file name in the *File Name* text box for the first of the images (or select an icon for an existing file to overwrite it). MetaFluor will append the number (1, 2, etc.) to the end of the file name to indicate the wavelength. If the desired folder name is not listed at the top of the dialog box, use the *Save In* list or Up One Level button to locate the correct drive and folder. Choose *Save* to close the dialog box.
5	If you chose an existing file name, a dialog box will inform you that the file already exists. Select *No* if you want to select another file name. Otherwise, Select *Yes.*

Loading Reference Images (External Monitor Display)

To load a set of reference images, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Reference Images. The Reference Images dialog box will appear.
2	To load a background image or pair of images, choose *Load from Disk* below the *Background References* text label. The Load Background dialog box will appear.
3	Choose *Select File*. A file selection dialog box, also entitled Load Background, will appear.
4	Select the desired image file. If the appropriate folder name is not listed at the top of the dialog box, use the *Look In* list of Up One Level button to change to the correct location. **AND** Choose *Open*. The file selection dialog box will close.
5	From the Load Background dialog box, choose *OK*. The Reference Images dialog box will reappear.
6	To load a shading correction (white reference) image or pair of images, choose *Load from Disk* below the *Shading References* text label. The Load Shading dialog box will appear.
7	Choose *Select File*. A file selection dialog box, also entitled Load Shading, will appear.
8	Select the desired image file. If the appropriate folder name is not listed at the top of the dialog box, use the *Look In* list of Up One Level button to change to the correct location. **AND** Choose *Open*. The file selection dialog box will close.
9	From the Load Shading dialog box, choose *OK*. The Reference Images dialog box will reappear.
10	Choose *Close.*

Reference Images - Dialog Box Options (External Monitor Display)

Subtract Backgrounds (playback mode only)

Enables background subtraction for the images being played back.

Load from Disk (Background, acquisition or playback mode)

Loads previously saved background reference images from disk.

Save to Disk (Background, acquisition mode only)

Saves the current set of background reference images acquired using the Experiment Control Panel command to disk.

Create Backgrounds

Creates artificial background images that consist of specified gray levels. You can select separate gray levels to use for each wavelength. This option is useful for subtracting an offset from each image. When you use this command, it replaces the last pair of background reference images acquired using the Experiment Control Panel with the new artificial background images.

Correct Shading (playback mode only)

Enables shading correction for the images being played back.

Load from Disk (Shading, acquisition or playback mode)

Loads previously saved white (shading) reference images from disk.

Save to Disk (Shading, acquisition mode only)

Saves to disk the current set of white (shading) reference images acquired using the Experiment Control Panel.

View Backgrounds and References (playback mode only)

Saves to disk the current sets of backgrounds and white (shading) reference images acquired using the Experiment Control Panel.

Close

Closes the dialog box.

Reference Images - Computer Window Display (Run Experiment Menu)

Creates, loads, displays, saves, or discards background and shading reference images for correction of images being acquired or played back and displayed in image windows on the computer desktop. Enables background subtraction and shading correction. Creates artificial background images using specified gray levels.

Use this command to enable background subtraction and shading correction. You can acquire, load, display, save, or discard background reference images or white (shading) reference images. You can also use this command to create artificial background images, each with their own specified gray level value. You may want to do so when you need to subtract a different offset from each wavelength. You can disable background subtraction and/or shading correction by clearing the *Subtract Backgrounds* or *Correct Shading* check boxes.

The *Acquire* button in the *Background References* group of command buttons acquires a background reference image for all wavelength images for which you have selected *Image* from the *Background* drop-down lists. Similarly, the *Acquire* button in the *Shading References* group of command buttons acquires a shading correction image (also called a "white reference" image) for each wavelength image for which you have selected *Image* from the corresponding *Shading* drop-down list. The *View* buttons display the pertinent acquired images in image windows. When you choose a *View* button, it will switch to a *Hide* button. The image windows can be hidden using the *Hide* buttons.

Note: If you acquire reference images and then change the size of the acquisition region (Configure Acquisition command, Configure menu), MetaFluor will detect mismatches between the sizes of the reference and data images. Any mismatched reference images will be discarded, and a message box will be displayed that informs you of this.

Note: If you are acquiring images with a digital camera and have already acquired reference (background subtraction or shading correction) images, and you then change the binning setting in the **Configure Acquisition** dialog box, MetaFluor will check for mismatches between the binning setting of the reference and data images. If a mismatch is detected, any mismatched reference images will be discarded, and a message box will be displayed that informs you of this.

WARNING:
The *Discard* buttons delete the reference images from the temporary memory buffer that MetaFluor uses to store the reference images while you are working on them. If you simply don't want to see the reference images on your computer monitor, choose the *Hide* buttons instead.

Note: You will need to use a different **Reference Images** dialog box when displaying images on an external video monitor.

See Also:

Experiment Control Panel

Reference Images - External Monitor Display

Configuring Background and Shading Correction (Computer Window Display)

To configure background subtraction and shading correction, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Reference Images. The Reference Images dialog box will appear.
2	If you are configuring background subtraction for the image series, select the *Subtract Backgrounds* check box. **OR** If you only want to correct shading, skip to Step 4.
3	For each wavelength image, select the method of background subtraction you want to use from its corresponding *Background* drop-down list. Select *Image* if you want to use an image that you already have on disk or one that you are about to acquire. *Constant* if you want to simply reduce the entire image by a specific number of gray levels. When you select this option, a spin box will appear in the *Parameter* column, from which you can select the gray value. *Region* if you want to subtract the average gray value in a region of interest that you have defined on the image. When you select this option, a spin box will appear in the *Parameter* column, from which you can select the number corresponding to the region. (You must first define the regions.)
4	If you are configuring shading correction for the image series, select the *Correct Shading* check box. **OR** If you do not want to configure shading correction, skip to Step 7.
5	For each wavelength image, select the method of shading correction you want to use from its corresponding *Shading* drop-down list. Select *Image* if you want to use an image that you already have on disk or one that you are about to acquire, or *None* for wavelength images for which you do not want to perform shading correction.
6	If you selected *Image* for background subtraction (Step 3) or for shading correction

(Step 5), follow the procedure for **acquiring and saving new reference images** or for **loading previously stored reference images.**

7   When you have finished, choose *Close.*

Acquiring and Saving New Reference Images (Computer Window Display)

To acquire reference images for background subtraction or shading correction, use the following procedure.

Step	Action
1	If you are acquiring background subtraction images, set up your equipment to acquire the background reference images. Now continue to Step 2. **OR** If you are acquiring only shading correction images, skip immediately to Step 4.
2	Choose *Acquire* from the *Background References* command button group. MetaFluor will acquire background images for each wavelength image for which you have selected *Image* from the corresponding *Background* drop-down list. **Note:** You can choose the *View* button to display the background images in image windows on the computer monitor. The *View* button will change to a *Hide* button, which you can use to close the image.
3	To save the new background images, choose *Save* from the *Background Subtraction* option group. The Save Background dialog box will appear. Choose *Load File.* A file selection dialog box, also entitled Save Background, will appear. Type a name for the new image in the *File Name* text box and choose *Save.* The file selection dialog box will close. Now choose *OK* from the Load Background dialog box to return to the Reference Images dialog box will reappear.
4	If you are acquiring shading images, set up your equipment to acquire the shading reference images.
5	Choose *Acquire* from the *Shading References* command button group. MetaFluor will acquire background images for each wavelength image for which you have selected *Image* from the corresponding *Background* drop-down list. **Note:** You can choose the *View* button to display the shading images in image windows on the computer monitor. The *View* button will change to a *Hide* button, which you can use to close the image.
6	To save the new shading images, choose *Save* from the *Shading Correction* option

group. The Save Shading dialog box will appear. Choose *Load File.*

A file selection dialog box, also entitled Save Shading, will appear. Type a name for the new image in the *File Name* text box and choose *Save.* The file selection dialog box will close.

Now choose *OK* from the Save Shading dialog box to return to the Reference Images dialog box will reappear.

Loading Previously Stored Reference Images (Computer Window Display)

To load reference images for background subtraction and shading correction, use the following procedure:

Step	Action
1	If you are loading a set of background subtraction images, choose *Load* from the *Background References* command button group. The Load Background dialog box will appear.
2	Choose *Load File.* A file selection dialog box, also entitled Load Background, will appear.
3	Select the desired background reference image set. If necessary, use the *Look In* list or Up One Level button to change the current directory location to the correct folder. **AND** Choose *Open.* The background reference image set will be loaded and the file selection dialog box will close.
4	From the Load Background dialog box, choose *OK.* The Reference Images dialog box will reappear.
5	To load a shading correction (white reference) image set, choose *Load* from the *Shading References* command button group. The Load Shading dialog box will appear.
6	Choose *Load File.* A file selection dialog box, also entitled Load Shading, will appear.
7	Select the desired shading reference image set. If necessary, use the *Look In* list or Up One Level button to change the current directory location to the correct folder. **AND** Choose *Open.* The shading reference image set will be loaded and the file selection dialog box will close.
8	From the Load Shading dialog box, choose *OK.* The Reference Images dialog box will reappear.

Reference Images - Dialog Box Options (Computer Window Display)

Subtract Backgrounds

Enables background subtraction. Clear this check box to disable background subtraction.

Correct Shading

Enables shading correction. Clear this check box to disable shading correction.

Wavelength

This column indicates the wavelength image for which you are configuring background subtraction and/or shading correction.

Background

Selects the method of background subtraction you want to use:

Image uses an image that you already have on disk or one that you are about to acquire.

Constant will subtract the number of gray levels specified by the *Parameter* spin box from each pixel in the wavelength intensity image.

Region subtracts the average gray value in the region specified by the *Parameter* spin box from each pixel in the wavelength intensity image*.*

None disables background subtraction for just the corresponding wavelength image.

Parameter (Backgrounds group)

The items that appear in this field will depend on the *Background* selection for the corresponding wavelength:

If you selected *Image,* static text will be displayed, indicating either *(In memory)* if an image has been loaded, or *(No image)* if the image has not yet been loaded.

If you selected *Constant,* a spin box will be displayed, from which you can select a gray value to be subtracted from each pixel in the wavelength intensity image.

If you selected *Region,* a spin box will be displayed, from which you can select the number corresponding to the region of interest whose average gray value will be subtracted from each pixel in the wavelength intensity image.

If you selected *None,* static text indicating *"n/a"* will be displayed.

Shading

Selects the method of shading correction you want to use:

Image uses an image that you already have on disk or one that you are about to acquire.

None disables shading correction for just the corresponding wavelength image.

Parameter (Shading group)

The items that appear in this field will depend on the *Shading* selection for the corresponding wavelength:

If you selected *Image,* static text will be displayed, indicating either *(In memory)* if an image has been loaded, or *(No image)* if the image has not yet been loaded.

If you selected *None,* static text indicating *"n/a"* will be displayed.

Acquire (Background References)

Acquires a background reference image for each wavelength image for which you selected *Image* from the corresponding *Background* drop-down list.

Load (Background References)

Loads a set of previously saved background reference images.

View (Background References)

Displays the acquired or loaded background images in separate desktop image windows. When you choose this button, its label will change to *"Hide."*

Hide (Background References)

Hides the background images from view. This is useful when you want to minimize the number of image windows open on your workspace but do not want to discard the background images.

Save (Background References)

Saves the newly acquired background images to disk.

Discard (Background References)

Deletes the background images from the temporary memory buffer that MetaFluor uses to store reference images while you are working on them. If you simply do not want to see the reference image on your workspace, you should choose *Hide.*

Acquire (Shading References)

Acquires a shading reference image for each wavelength image for which you selected *Image* from the corresponding *Shading* drop-down list.

Load (Shading References)

Loads a set of previously saved shading reference images.

View (Shading References)

Displays the acquired or loaded shading images in separate desktop image windows. When you choose this button, its label will change to *"Hide."*

Hide (Shading References)

Hides the shading images from view. This is useful when you want to minimize the number of image windows open on your workspace but do not want to discard the shading images.

Save (Shading References)

Saves the newly acquired shading images to disk.

Discard (Shading References)

Deletes the shading images from the temporary memory buffer that MetaFluor uses to store reference images while you are working on them. If you simply do not want to see the reference image on your workspace, you should choose *Hide.*

Close

Closes the dialog box.

Select Save Region (Run Experiment Menu)

Defines the image area that you want to save when images are acquired.

Use this command when you only need to save a small subregion of the images or when you have limited storage space. Saving a selected part of acquired images is faster than saving entire images.

Before defining the Save Region, you will be asked to select the source image that you want to use for defining the region. The other images will be closed temporarily while you define the Save Region. You should acquire one set of images before you define the region, so that you will know where to place the boundaries of the region. You can define the region using the Rectangular Region, Ellipse Region, Trace Region, or Auto-Trace Region Tool in the Region Toolbar.

If you want to save the entire image, rather than a previously selected Save Region, you can do so by using this command without drawing a region. A message will appear, stating that, since you did not define a region, the entire image will be used.

Selecting a Save Region

To select a save region for image acquisition, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Select Save Region. The Select Source Image for Defining Region dialog box will appear.
2	Choose *Acquire Images* to acquire one set of images. It is important to acquire a set of images now, so that you will know where to define the region of interest; otherwise, the image display will be the last image in the video board's memory--which could be its test pattern image.
3	Select the image that you think is best suited for defining the save region from the *Source Image* group. Depending on the dye and other experimental conditions, one image may be better than the others.
4	Choose *OK* to continue. **OR** Choose *Cancel* to cancel the Select Save Region command.
5	MetaFluor will close all image windows and dialog boxes temporarily, except for that of the image selected in Step 3. It will open the Region Toolbar and the Define Save Region dialog box.
6	Using the Rectangular Region Tool, define the region of the image you want to save. **Do not define more than one region.** You can move and resize the region using the Locator Tool. You can choose *Clear Region from Image* to remove the defined region from the image. **OR** Choose *Load Region from Disk* if you want to use a region that you have saved to disk. Select the icon for the desired file from the Load Region File dialog box and choose *Open.*
7	If you want to save the defined region for later use, choose *Save Region to Disk.* Type a file name for the region file in the *File Name* text box and choose *Save.*
8	After you have finished editing or defining the region, choose *Done Defining Save Region.* MetaFluor will close the Region Toolbar and the Define Save Region dialog box and restore

the image windows and dialog boxes that were
open prior to editing the regions.

Select Source Image for Defining Regions - Dialog Box Options

Image

Selects the image to be used for defining the regions of interest that are to be used for defining regions of interest for graphing data to the measurements graphs. Depending on the dye and other conditions, one image may be better than the others.

Acquire Images

Acquires one set of images. If you are about to acquire images and want to perform measurements during your experiment, you should use this command before defining the regions, so that you will know where to place the regions. Otherwise, the image displayed will be the last image in the video board's memory--which could be its test pattern image.

OK

Closes all image windows and dialog boxes except for the selected image and opens the Edit Regions dialog box so that you can define the regions.

Cancel

Cancels the Define Regions for Measurement command.

Define Save Region - Dialog Box Options

Load Region from Disk

Loads a region file that has been saved to disk (using *Save Regions to Disk).* This option opens the Load Region File dialog box. Select the icon for the desired file. If necessary, use the *Look In* drop-down list box or the Up One Level icon button to select the appropriate drive and folder. Choose *Open.* The saved region will appear on the image.

Save Region from Disk

Saves the region that is currently defined on the image to disk. This option opens the Save Region File dialog box. Select the destination drive and folder for the log file using the *Save In* drop-down list or Up One Level icon button. Then type the desired file name in the *File Name* text box. Choose *Save* to save the region.

Clear Region from Image

Clears the current region from the image. Use this option if you want to use the entire image.

Print with Region Outlines

Prints the image with the region of interest outline superimposed on the image. If there is no region of interest defined on the image, this command simply prints the image. Select the color to be used for printing the outline using *Stamp Color.* Type the desired title for the printed image in the *Image Title* text box. Choose *Print,* and the standard Print dialog box will appear. Select the desired printing options and choose *OK.*

Done Defining Save Region

Use this command when you are done defining the Save Region. MetaFluor will close the Region Toolbar and the Define Save Region dialog box. It will also restore the image windows and dialog boxes that were open prior to editing regions.

Event Marks (Run Experiment Menu)

Marks the time at which a significant event occurred.

Use this command when you want to record a significant event during the experiment in a log file. For example, you may want to record the time that you added a receptor agonist to your perfusion medium. You can use the Event Marks command to annotate your data log with the desired text. Each text line will have the current graph time associated with it. If you are saving images to play back later, MetaFluor will also record the event in the .inf file associated with the images so that it can display the event text at the appropriate time as you play back the images.

The Event Marks dialog box can be expanded and condensed using the *More >>* and *Less <<* command buttons. When the dialog box is in its expanded form, you can compose text for the event mark in the *Event Text* box. Additional options will be available for configuring the use of the experimental timer. Use the *Mark New Event Now* command button to log newly created events immediately. Events can be added to the Stored Events List using the *Add New Event to List* command button. After events have been added to the Stored Events List, you can log stored events from the dialog box in its condensed form, either by double-clicking the entry in the Stored Events List, by choosing the *F5: Mark* command button, or by pressing the [F5] function key on your keyboard.

Adding predetermined events to the Stored Events List before starting is usually the best approach to use for a new experiment with fast acquisition intervals. You can then log the event marks as needed during the experiment. If you choose this approach, you will be able to condense the dialog box using *Less <<* after you have added your events to the list; all the commands you need to log stored events will still be available in the condensed dialog box.

Note: If you find that logging events is a little tricky, it may be that the *Timelapse* interval you selected using *Timed Acquisition* (Experiment Control Panel) is too short. A very short interval, combined with a large number of *Frames to Average* (Experiment Control Panel), may not allow MetaFluor enough time to handle image acquisition and other tasks, such as logging events.

See Also:

Experiment Control Panel:

For Video Camera with External Monitor

For Video Camera with Computer's Monitor

For Digital Camera

For Playback

Logging One Event

If you have just one event to log and a long acquisition interval, use the following method to log the event:

Step	Action
1	Enable data logging in the Experiment Control Panel dialog box by selecting *Log Data.*
2	From the Run Experiment menu, choose Event Marks. The Event Marks dialog box will appear. If the dialog box is not expanded, choose *More* >> so that you can see all of the options.
3	If you want to insert a delay between the time you trigger the event mark and when it is actually marked, select *Enable Timer* and then enter a delay time in the *Count-Down Timer* spin box.
4	If you want to have audible feedback that your event mark was logged or marked, select *Beep on Event.*
5	Type the desired text in the *Event Text* box.
6	If you want to run a journal when the event is marked, choose the *Journal to Be Executed When Event Is Marked* command button (labeled *"Select").* The Select a Journal to Run dialog box will appear.
	AND Select the file for the journal you want to run and choose *Open.*
7	When you want to mark this particular event during the experiment, choose *Mark New Event Now.* MetaFluor will note that the event has been logged by displaying "Logged" next to the *Event Text* static text or "Marked" to indicate that it received the event but there was no log file open and enabled.
	If you associate a timed delay with the event mark, the clock will count down from the moment you choose *Mark Now* until the time is reached, and then will start counting upwards from that time (indicated by a "+").
	WARNING: Image acquisition tasks have priority over confirmation of event marks (particularly when using short acquisition intervals), so you may not immediately see "Logged" next to *Event Text.* Choosing *Mark Now* successively will cause multiple events to be logged in the log file.
8	If you want to add the event text to the list of stored events, choose *Add New Event to List.*

Creating a Stored Events List

To create stored events for the Stored Events List, use the following procedure:

Step	Action
1	Enable data logging in the Experiment Control Panel by selecting *Log Data.*
2	From the Run Experiment menu, choose Event Marks. The Event Marks dialog box will appear. If the dialog box is not expanded, choose *More >>* so that you can see all of the options.
3	If there are events from a previous work session that you do not want to use, choose *Clear* to clear all entries from the Stored Events List. **OR** Delete any unwanted events by selecting them from the Stored Events List and choosing *Delete.*
4	In the *Event Text* box, type the desired text for the first event you want to use during the experiment.
5	If you want to insert a delay between the time you trigger the event mark and when it is actually marked, select *Enable Timer* and then enter a delay time in the *Count-Down Timer* spin box.
6	If you want to have audible feedback that your event mark was logged or marked, select *Beep on Event.*
7	If you want to run a journal when the event is marked, choose the *Journal to Be Executed When Event Is Marked* command button (labeled *"Select").* The Select a Journal to Run dialog box will appear. **AND** Select the file for the journal you want to run and choose *Open.*
8	Choose *Add New Event to List.* The event text will appear in the Stored Events List.
9	Repeat Steps 4 - 8 for each new event you want to use during the experiment. If you need to change the order in which the event marks appear in the list, highlight the event you want to move and choose the appropriate *Move* button. If you want to condense the dialog box to conserve space, choose *Less <<* after you have finished adding to the list.

Using the Stored Events List

To use the stored events list during an experiment to log events, use the following procedure:

Step	Action
1	Create a **Stored Events List.**
2	Select the first event in the Stored Events List that you are likely to use during the experiment. Keep the Event Marks dialog box open and visible on your computer screen.
3	Using the Experiment Control Panel, enable logging by selecting *Log Data,* and start the experiment when you are ready by choosing *Acquire.*
4	When you want to log the selected event mark, choose *F5: Mark,* press the [F5] key, or double-click the entry in the Stored Events List. MetaFluor will note that the event has been logged by displaying "Logged" next to the *Status* text or "Marked" to indicate that it received the event but there was no log file open and enabled.

If you associate a timed delay with the event mark, the clock will count down from the moment you choose *Mark Now* until the time is reached, and then will start counting upwards from that time (indicated by a "+").

WARNING:
Image acquisition tasks have priority over confirmation of event marks (particularly when using short acquisition intervals), so you may not immediately see "Logged" next to the *Status* text. Choosing *F5: Mark* successively will cause multiple events to be logged in the log file.

5	Repeat Step 4 as needed throughout the experiment.

Event Marks - Dialog Box Options

Stored Events List

Lists event names for event text that you have already composed so that you can quickly select and log desired events at any time during the experiment. If a journal has been associated with the event, the entry will list the name of the journal in parentheses. Similarly, if the event has a delay associated with it the time, in seconds, will be listed in parentheses.

F5: Mark

Logs the selected event in the Stored Events List to the open log file and indicates that it was successful by displaying "Logged" next to the *Status* text. If there is no log file open, "Marked" will be displayed instead. Double-clicking the desired event in this list will cause the event to be selected and logged simultaneously. If you associate a timed delay with the event mark, the clock will count down from the moment you choose *Mark Now* until the time is reached, and then will start counting upwards from that time (indicated by a "+"). If you mark an event that does not have a timer associated with it while the timer for another event is still running, the act of marking the untimed event will clear the timer.

F6: Next

Selects the next entry in the Stored Events List. If the last entry is currently highlighted, the first entry in the list will be selected.

Delete

Deletes the selected event from the Stored Events List.

Move

Moves the highlighted event mark entry up or down in the list.

Clear

Clears all events from the Stored Events List.

Load

Loads a set of event marks that has been previously stored on disk in an .evt file.

Save

Saves the current set of event marks as an .evt file.

More >>

Expands the dialog box.

Less <<

Condenses the dialog box.

Status

Indicates the status of the last event mark to be marked or logged.

Reset

Resets the timer and clears the timer display.

Beep on Event

Issues the computer's beep sound when an event is logged.

Enable Timer

Enables and disables the use of the experimental timer for the event mark being configured.

Event Text

Use this text box to compose the text for the event.

Count-Down Timer

When you add an event to the Stored Event List, you can associate it with a timer. To do so, type the text in the *Event Text* box and enter the timer value (in seconds) in the *Count-Down Timer* spin box; then choose *Add New Event to List.* In the Stored Events List, you will see the event with the time next to it. For example, if the event is "Test" and the time is "10" you will see "Test (10)." After you click the event, the timer will start counting (this happens regardless).

Journal to Be Executed When Event Is Marked ("Select")

Opens the Select a Journal to Run dialog box, from which you can select a journal that will run when the associated event is marked.

Mark New Event Now

Immediately logs the event text displayed in the *Event Text* box. Use this logging command when you want to log a single event without adding and logging an event from the Stored Events List.

Add New Event to List

Adds the event displayed in the *Event Text* box to the Stored Events List.

Close

Closes the dialog box.

Event Mark Hotkeys (Run Experiment Menu)

Provides shortcut keys to generate the event marks defined in the Event Marks dialog box.

Use this command when you want to use a shortcut key to generate an event mark already defined in the Event Marks dialog box.

The Event Mark Hotkeys command opens a secondary menu consisting of two commands and keyboard shortcuts for the first ten events in the *Stored Events* list in the Event Marks dialog box.

The Mark Current Event command generates an event mark that is equivalent to the one currently selected in the *Stored Events* list.

The Move to Next Event command selects the next event in the *Stored Events* list as the current event. However, it does not generate an event mark.

The remaining ten menu items in the secondary menu associate the keyboard shortcut keys [SHIFT] + [F1] through [SHIFT] + [F10] with the first ten events in the *Stored Events* list. Pressing the appropriate keyboard shortcut keys generates the corresponding event mark and selects that event mark as the current event. Menu items that have been assigned to an event mark will appear in the menu with the name of the event mark; unassigned items will be named *"Mark Event #n,"* where *n* indicates the number of the item in the list.

You do not need to have the Event Marks dialog box open to use these shortcut keys. However, you may want to keep the dialog box open so that you can see which event mark is the currently selected one.

Shortcuts:

Mark Current Event = [F5]

Move to Next Event = [F6]

First ten events = [SHIFT] + [F1] through [SHIFT] + [F10]

See Also:

Event Marks

Using Event Mark Hotkeys

To use the Event Mark Hotkeys, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Event Mark Hotkeys. A secondary menu will appear. The menu lists the event numbers and the keyboard shortcut assigned to each event.
2	To mark a particular event, choose it from the secondary menu. **OR** Use the keyboard shortcut listed next to the journal: press and hold the [SHIFT] key. Then press the assigned function key.
3	If you do not want to use the individual shortcuts listed, you can use the Move to Next Event command (F6) to move to the next event in the list. Then use the Mark Current Event command (F5) to mark the newly selected event. **Note:** The Move to Next Event command does not "wrap" to the top of the list. Thus, you will need to select the first event from the Event Marks dialog box or use its shortcut if you want to repeat the sequence of events.

Show Event List (Run Experiment Menu)

Displays a list of the time and text of all stored events during playback of the current experiment.

Use this command to see an experiment's stored events during playback. You can also use this command to load the set of images associated with an event by clicking that event in the Event List dialog box.

Note: You can select whether or not to have the dialog box appear every time an event mark occurs during playback by enabling or disabling *Display Dialog When an Event Mark Occurs During Playback* from the Playback Preferences dialog box (Preferences command, File menu).

This command is available only during playback of a previously stored experiment.

See Also:

Event Marks

Preferences

Showing the Event List

To display the Event List, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Show Event List. The Event List dialog box will appear.
2	As you play the experiment forward or in reverse using the controls in the Experiment Control Panel, MetaFluor will highlight the current event in the Event List.
	To go to a particular event, select the event name from the Event List dialog box so that it is highlighted, and choose *Go to Event.* (Or you can double-click the event name to go to the event.)
3	Choose *Close* when you have finished.

Event List - Dialog Box Options

Events from This Experiment

Lists the time and text of all stored events for the experiment. The units of time displayed in the Event List are the same as those set in the Experiment Control Panel.

Go to Event

Displays the images associated with the event currently selected in the *Events from This Experiment* list. You can use this option to display an event and its associated images rather than playing through the experiment using the Experiment Control Panel.

Close

Closes the dialog box.

Stream Setup (Run Experiment Menu)

Configures the acquisition settings used for the Acquire Stream command.

Use this command before you use the **Acquire Stream** command to specify the acquisition wavelengths, number of frames to acquire, and the camera settings. After you define the Stream Setup and **Configure Acquisition** settings, you can use the Stream Acquisition command to acquire the specified number of frames as fast as possible into computer memory. When all of the frames have been acquired, MetaFluor will transfer the image data into the experiment, performing the wavelength or ratio image saving, and data logging tasks (which are specified in the **Experiment Control Panel - Digital Camera** before you carry out the Stream Acquisition command). If you have a Physik piezo focuser and the appropriate acquisition hardware (see *Hardware*, below), you can also use the Stream commands to perform Z-streaming.

You can configure your stream setup to run a journal before and after acquisition. This is particularly useful in situations where you want to initiate acquisition when the system receives a TTL pulse signal. In this example, the "before" journal can be a **trigger journal,** and the "after" journal can be one that resets the TTL trigger after acquisition has finished.

Note: This command is unavailable in the MetaFluor Offline system.

Hardware

The fast acquisition mode used by the Acquire Stream command is currently supported by the following video hardware:

All Photometrics cameras,

All Princeton Instruments cameras,

All video cameras connected to the Flashbus video acquisition board, and

All cameras (video and digital) connected to the MuTech video acquisition board.

Two-wavelength streaming is currently supported only by the Princeton Instruments PentaMax or MicroMAX camera, or by the MuTech board in combination with a monochrome camera-- and then only if you are using a Sutter Lambda 10, Lambda 10-2, or DG4 filter wheel, or a Polychrome monochromator as your illumination device.

WARNING: If you attempt to use a streaming exposure time that is shorter than the fastest readout time your camera can handle, your actual exposure time will be limited by the readout time. If this occurs, MetaFluor will display a warning message.

See Also:

Acquire Stream

Experiment Control Panel - Digital Camera

Configure Acquisition

Trigger Journals

Configuring Stream Setup

To configure the Stream Setup command, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Stream Setup. The Stream Setup dialog box will appear.
2	From *Number of Frames to Acquire,* select the number of image frames you want to acquire.
	MetaFluor will display the amount of memory the stream will use (based on the size and number of images that you want to acquire). It will also display the amount of memory available. This information will help you determine how many frames you can acquire in one stream.
3	From the *Number of Wavelengths* option button group, select the number of wavelengths that you want to acquire in stream mode.
	Note: To perform dual-wavelength stream acquisition, you must have a Sutter Lambda 10, Lambda 10-2, or DG4 filter wheel or a Polychrome monochromator, and either a monochrome camera connected to a MuTech video acquisition board or a Princeton Instruments PentaMax camera.
4	Select the desired wavelength image for acquisition of the first stream from the *First Wavelength* list. If you are conducting single-wavelength stream acquisition, this will be the only wavelength acquired.
	AND If you are conducting a dual-wavelength stream acquisition, select the desired wavelength image for acquisition of the second stream from the *Second Wavelength* list.
5	If you are using a "video rate" camera, skip to Step 9.
	OR If you are using a digital camera, continue to Step 6.
6	Select the desired state for the camera from the *Camera State* list. The options available will vary depending on the camera used. The suggested setting for the PVCam camera is *HALT, CLEAR.*
7	If you are using an external shutter, select a shutter state from the *Shutter Mode* list: *Open for Expose, Always Closed,* or *Always Open.*

8 Select the desired clear mode for the camera from the *Clear Mode* list.

The options available will vary depending on the camera used. The suggested setting for a frame transfer camera operating in the FT mode is *CLEAR PRE EXPOSURE.*

9 If your system includes a DVP board, the *Destination* radio button group will be enabled. To take advantage of the high-speed, real-time streaming of acquired frames to hard disk, select *Stream to Real-Time Hard Disk.*

OR
To use the default method of acquiring the entire stream to RAM and processing the images to disk afterwards, select *Stream to RAM.*

10 If you want to run a journal (e.g., a trigger journal) before and after stream acquisition, choose the *Before Streaming* command button and select the journal to run before acquisition from the Select a Journal dialog box. Then choose *Open* to return to the Stream Setup dialog box.

AND
Choose *After Streaming* and select the journal to run after acquisition from the Select a Journal dialog box. Then choose *Open* to return to the Stream Setup dialog box.

11 When you have finished configuring the stream setup, choose *OK.*

Stream Setup - Dialog Box Options

Number of Frames to Acquire

Specifies the number of frames to acquire. As you change this value, the value displayed by the *Amount of Memory Stream Will Use* will be updated.

Your Current Acquisition Region Is

Displays the size of the acquisition region. (The acquisition region is defined in the **Configure Acquisition** dialog box.)

Your Current Exposure Time Is

Displays the current exposure time (digital) or number of frames (video) for the selected wavelength image.

Amount of Memory Available

Displays the amount of memory available for use by the stream during acquisition.

Amount of Memory Stream Will Use

Displays the amount of memory needed to complete the stream acquisition. If the amount of memory used by the stream exceeds the total amount of free memory, acquisition will not be possible. When this situation occurs, a message will be displayed on the *Status* line.

Estimated Total Exposure Time

Displays what the total exposure time (digital) or total number of acquired frames (video) will be for the stream acquisition, based on the selected *Number of Frames to Acquire* and the exposure time setting.

Camera State

Specifies the camera state used during acquisition. The options available will vary depending on the camera used. The suggested setting for the PVCam camera is *HALT, CLEAR.* This option will be available only for acquisition with a digital camera.

Shutter Mode

Specifies the shutter mode to use during acquisition: *Open for Expose, Always Closed,* or *Always Open.* This option will be available only for acquisition with a digital camera.

Clear Mode

Specifies the mode used to clear the camera chip. The options available will vary depending on the camera used. The suggested setting for a frame transfer camera operating in the FT mode is *CLEAR PRE SEQUENCE.* This setting clears the chip prior to starting the first exposure. If you do not have a frame transfer camera, you should set this option to *CLEAR PRE EXPOSURE.* This setting clears the chip before each frame is exposed. This option will be available only for acquisition with a digital camera.

Number of Wavelengths

Specifies the number of wavelengths *(One* or *Two)* to acquire in the stream. To perform two-wavelength stream acquisition, you must have a Sutter Lambda 10, Lambda 10-2, or DG4 filter wheel or a Polychrome monochromator, and either a monochrome camera

connected to a MuTech video acquisition board or a Princeton Instruments PentaMax camera.

First Wavelength

Specifies the wavelength image to be used for the stream acquisition.

Second Wavelength

Specifies the wavelength image to be used for acquisition of the second stream of a dual-wavelength protocol.

Destination

If you have a DVP board, this option allows you to select between (1) the default method of acquiring frames rapidly to RAM and then processing the images afterwards on disk *(Stream to RAM),* and (2) acquiring the image frames directly to disk by high-speed streaming *(Stream to Real-Time Hard Disk).*

Before Streaming

Opens the Select a Journal dialog box, from which you can pick a journal that will run before the actual stream acquisition. For example, you may want to run a Trigger Journal that puts the system into a standby mode until a TTL trigger is received, at which point the stream acquisition will proceed.

After Streaming

Opens the Select a Journal dialog box, from which you can pick a journal that will run after the stream acquisition has completed. For example, you may want to run a journal that resets the TTL trigger.

Status

Displays a message regarding the status of the stream acquisition and alerts you to any problems that may occur.

OK

Configures the stream acquisition.

Cancel

Cancels the command.

Acquire Stream (Run Experiment Menu)

Acquires images from one or two wavelengths as rapidly as possible into computer RAM memory.

Use this command to perform high-speed acquisition of images. The number of wavelengths and images to be acquired is defined by the Stream Setup command. If you have a Physik piezo focuser and the appropriate acquisition hardware (see *Hardware*, below), you can also use the Stream commands to perform Z-streaming. After the images have been acquired into RAM, MetaFluor will transfer the image data to disk.

Before using this command, you must use the **Stream Setup** command to specify the wavelength, number of frames to acquire, the camera state, the shutter mode, and the clear mode. You must also use the **Configure Acquisition** command to define other acquisition settings.

Note: This command is unavailable in the MetaFluor Offline system.

Hardware

The fast acquisition mode used by the Acquire Stream command is currently supported by the following video hardware:

> All Photometrics cameras,

> All Princeton Instruments cameras,

> All video cameras connected to the Flashbus video acquisition board, and

> All cameras (video and digital) connected to the MuTech video acquisition board.

Two-wavelength streaming is currently supported only by the Princeton Instruments PentaMax or MicroMAX camera, or by the MuTech board in combination with a monochrome camera-- and then only if you are using a Sutter Lambda 10, Lambda 10-2, or DG4 filter wheel, or a Polychrome monochromator as your illumination device.

WARNING: If you attempt to use a streaming exposure time that is shorter than the fastest readout time your camera can handle, your actual exposure time will be limited by the readout time. If this occurs, MetaFluor will display a warning message.

See Also:

Stream Setup

Experiment Control Panel - Digital Camera

Configure Acquisition

Using Stream Acquisition

To acquire a high-speed stream of images, use the following procedure:

Step	Action
1	Configure the stream acquisition using the **Stream Setup** command.
2	Select the Run Experiment menu.
3	Choose Acquire Stream and wait while the camera acquires the images.
	When all of the images have been acquired and stored in memory, MetaFluor will perform the image saving and data logging tasks as configured in the **Experiment Control Panel - Digital Camera** dialog box.

Move All Regions (Run Experiment Menu)

Moves all regions of interest up, down, left, or right in increments of 1 or 10 pixels.

Use this command to move regions at any time (including during image acquisition) while completing an experiment. Move All Regions is ideal for situations in which all of the objects that you are measuring have moved simultaneously and in the same direction. For example, if you add a solution to a dish, the influx of solution may shift the field of view by a number of pixels. Move All Regions allows you to shift all of the regions of interest to follow the objects you were measuring.

MetaFluor does not record region moves in log files. If you need to know when you moved regions, you should create an event mark and log that whenever you move regions. Alternatively, you can assign a journal to a journal toolbar button that runs the Write to Log File journal function, and enter text that indicates the change that was made.

WARNING:
If you move or remove a region while graphing the data, all data in the graphs will be erased.

See Also:

Event Marks

Moving All Regions

To move all regions, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Move All Regions. The Move All Regions dialog box will appear.
2	To move the regions in increments of one pixel, use the buttons surrounding the *+/- 1* text to move the regions in the desired direction. Repeat the commands as necessary.
3	To move the regions in increments of ten pixels, use the buttons surrounding the *+/- 10* text to move the regions in the desired direction. Repeat the commands as necessary.
4	Choose *OK* when you have finished.

Move All Regions - Dialog Box Options

Up

Moves all regions up in increments of either one pixel or ten pixels.

Down

Moves all regions down in increments of either one pixel or ten pixels.

>>

Moves all regions right in increments of either one pixel or ten pixels.

<<

Moves all regions left in increments of either one pixel or ten pixels.

Region Display Box

Displays the overall location of the entire collection of regions on the images. The box represents the perimeter of all the regions, not just of one region. You can drag the box inside this display to move the regions, rather than using the other dialog box options.

OK

Closes the Move All Regions dialog box.

Align Wavelengths (Run Experiment Menu)

Aligns the images of one to five wavelengths along the X and/or Y image axis to compensate for refractive differences.

Use this command to correct for variations in image alignment caused by the differences in light transmission and refraction at different wavelengths. When using two or more wavelengths to visualize and analyze a ratio or to create an image stack, because of the different refractive properties of the wavelengths, the images might not align correctly, producing false data in the analysis. To compensate for the refractive differences, you can determine the amount of image offset correction that needs to be applied to each wavelength, and have MetaFluor apply that correction to each image of the associated wavelength either immediately following image acquisition, or during image playback. The *Align Wavelengths* dialog box enables you to apply separate correction values to each wavelength for a maximum of five wavelengths. You can either type the X and Y values into the X and Y boxes, or use the position control arrows in the Adjust box to change the image position along the X and/or Y axis. You can also choose whether to apply the alignment correction values immediately after acquisition or during playback.

Aligning Wavelengths

To align two to five wavelengths, complete the following procedure.

Step	Action
1	Before starting the wavelength alignment steps, open the experiment images that you want to use to make your alignment settings or begin to acquire experiment images. You need an experiment that contains images from at least two different wavelengths. Use the ratio image of the two wavelengths to view and determine the alignment of the wavelengths.
2	From the *Run Experiment* menu, click *Align Wavelengths*, the Align Wavelengths dialog box opens.
3	In the *Wave* box, select the wavelength to which you want to apply the alignment settings. If you are acquiring only two wavelengths, you need to apply alignment settings to only one wavelength.
4	If you are applying the alignment settings to your wavelengths immediately after acquisition, click *Align after Acquisition*; or, if you are applying alignment settings during image playback, click *Align during Playback*.
5	In the *Adjust* box, click the left or right arrows to move the selected wavelength's image left or right. Click the up or down arrows to move the image up or down. The values in the X and Y boxes will change accordingly. *OR* If you know the values to apply to X and Y, type them into the X and Y boxes.
6	Repeat steps 3 through 5 until all wavelengths are aligned.
7	After all settings for each wavelength are complete, click *Close*.

Align Wavelengths - Dialog Box Options

Wave

Selects the wavelength to which you want to apply the adjustment.

Adjust

Adjusts the position of the image along the X and/or Y axis. Also, resets both the X and Y axis values to zero using the center button. Use the arrow buttons to change X and/or Y values. The left and right arrows change X; the up and down arrows change Y. The center button marked "0" resets both X and Y to zero. The selected values are displayed in the X and Y boxes.

X

Specifies the X axis offset value that you want to apply to images of the selected wavelength. Type the value into the box or click the left and/or right arrows in the *Adjust* box. This can be either a negative or positive value. A negative value moves the image to the left; a positive values moves the image to the right.

Y

Specifies the Y axis offset value that you want to apply to images of the selected wavelength. Type the value into the box or click the up and/or down arrows in the *Adjust* box. This can be either a negative or positive value. A negative value moves the image down; a positive values moves the image up.

Align after acquisition

Applies the wavelength alignment settings immediately after acquisition of the image. The applied value is stored with the image, preventing the image from being aligned a second time during playback.

Align during playback

Applies the wavelength alignment settings to images from the specified wavelengths during playback.

Close

Closes the Align Wavelengths dialog box.

Edit Conditions (Run Experiment Menu)

Defines up to five experimental conditions which can be used to "tag" the experimental data at appropriate times.

Drop-in: COND

Use this command to define experimental conditions (e.g., "staurosporine present") which can be used to characterize the state of the preparation during data acquisition or playback. These conditions will appear in the dialog box for the Conditions command, which contains a button that you can toggle on and off for each condition. This allows you to apply a numeric "tag" to the data as they are acquired. When you log the data, the state of each condition will appear in a separate column in the measurements file. If, for example, you use the default values for the ON and OFF states, a "0" will signify the OFF condition and a "1" will signify ON. You can specify a different tag for the ON and OFF conditions if you wish. With the Edit Conditions command, you will select both a descriptive name (e.g., "Stimulus applied") and a short name that will be used in the log file (e.g., "stim").

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Conditions

Editing Experimental Conditions

To define a set of experimental conditions, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Edit Conditions. The Edit Conditions dialog box will appear.
2	With the *Number of Conditions* spin box, select the number of different conditions that you want to use to "tag" the experimental data. You may select up to five conditions.
3	Type a descriptive name for the first condition in the *Condition Name* text box in the first row. This name will appear on a corresponding toggle button in the Conditions dialog box.
4	In the *Log Name* text box, type a corresponding short name that will be used as a column heading in the measurements file.
5	The default tags for the ON and OFF states are "1" and "0", respectively. If you want to use different values, type the values you prefer in the *ON Value* and *OFF Value* text boxes.
6	If you want to specify a numeric format for the tags, type the format string in the *Log Format* text box. For example, "##.#" will specify a format for numbers between 0 and 99, with one place after the decimal point.
7	Repeat Step 3 - 6, as necessary for each subsequent condition, using the text boxes in the pertinent rows.
8	When you have finished, choose *OK.*

Edit Conditions - Dialog Box Options

Number of Conditions

Selects the number of separate conditions that you want to define. A row of text boxes *(Condition Name* and *Log Name)* will become available for each condition.

Condition Name

Specifies a descriptive name (e.g., "Staurosporine added") for the condition. This name will appear on a corresponding toggle button in the Conditions dialog box.

Log Name

Specifies a short name (e.g., "staur") for the condition. This name will be used as a column heading in the measurements file when you log experimental data.

ON Value

Specifies a numeric tag for the ON state of the condition. The default value is *1.*

OFF Value

Specifies a numeric tag for the OFF state of the condition. The default value is *0.*

Log Format

Specifies a numeric format for the ON and OFF tags. For example, "##.#" will specify a format for numbers between 0 and 99, with one place after the decimal point.

OK

Accepts the current set of conditions and closes the dialog box.

Cancel

Rejects any newly defined conditions and closes the dialog box.

Conditions (Run Experiment Menu)

"Tags" experimental data, as they are acquired or played back, with up to five sets of experimental conditions.

Drop-in: COND

Use this command to "tag" your experimental data with a description of the state of the preparation during acquisition (e.g., "staurosporine present"). You will first need to define these conditions with the Edit Conditions command (Run Experiment menu). When you log the data, the state of each condition will appear in a separate column in the measurements file. If you used the default values in the Edit Conditions dialog box for the ON and OFF states, a "0" will signify the OFF condition and a "1" will signify ON.

This command offers a convenient way of rapidly labeling your data with up to five separate "flags" that describe the prevailing conditions of your preparation. This is somewhat similar to the use of the Event Marks command. However, the Conditions command will apply the condition "tag" continuously until you change its state (ON to OFF, or vice versa). Also, the Conditions dialog box will occupy far less space in the application window than the Event Marks dialog box.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Edit Conditions

Event Marks

Tagging Data with an Experimental "Conditions" State

To tag your experimental data with a conditional state during acquisition or playback, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Conditions. The Conditions dialog box will appear, with a toggle button for each condition you have defined with the **Edit Conditions** command (Run Experiment menu).
	Leave this dialog box open in your application workspace while you run the experiment.
2	At the appropriate times during data acquisition or playback, click the pertinent toggle button to "tag" all subsequent data. The color indicator next to the button will change in accordance with the state, light green for ON or dark yellow for OFF.
3	When you have finished acquiring or playing back your data, choose *Close.*

Conditions - Dialog Box Options

Condition Toggle Button

Tags data being acquired or played back with a "flag" that indicates the experimental condition. When you log the data, the state of each condition will appear in a separate column in the measurements file, with a "0" signifying OFF and a "1" signifying ON.

Condition Indicator

This colored box indicates the current state of the corresponding condition: light green for ON or dark yellow for OFF.

Close

Closes the dialog box.

Graphs Menu

Define Regions for Measurement (Graphs Menu)

Defines the regions of interest from which image data are to be measured, graphed in the time-based measurements graphs, logged to measurement files, or averaged for use in background subtraction. Opens the measurements graphs after regions of interest have been defined. Images can also be printed from this dialog box.

Use this command to define the regions of interest used for logging or graphing measurement data. You can also define a region from which an average grayscale value will be derived and used for background subtraction (selected in the Reference Images dialog box). Each measurement that is graphed or logged is an average of all of the pixels in its region of interest, after taking thresholding into account. If a region is used for background subtraction, its values will be set to 0, causing the trace to "disappear" off of the measurement graphs. This is done so that, if you change which region is to be used for background subtraction, the data in the measurements file will not become jumbled. Measurements cannot be computed by MetaFluor unless at least one region of interest has been defined using Define Regions for Measurement. You can also load and save regions to disk or clear regions from the image using this command.

Regions are defined using one of the Region Tools (Rectangular, Ellipse, Trace, or Auto-Trace Region Tool) in the Region Toolbar. The status line on the Region Toolbar displays the coordinates and size of the region that you are creating or editing. The coordinates for rectangular regions are expressed for the upper left corner. For elliptical regions, the coordinates of the centroid are given. For irregularly shaped regions, a **bounding rectangle** is placed over the region, and the coordinates of its upper left corner are given.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Before you define your regions of interest, you will be asked to select the source image (wavelength or ratio) that you want to use for defining the regions. If you are about to acquire images and want to perform measurements during your experiment, you will be able to acquire a single set of images now for use in defining your regions. If you are playing back stored images, you should select the image that you think is best suited for defining regions. Depending on the dye and other conditions, one image may be better than the others. The other images will be closed temporarily while you edit the regions. You should acquire one set of images prior to defining the regions so that you will know where to place regions.

As you define your regions, they will be numbered in the order you create them. The number will appear next to the region if you have selected the *Draw Labels Next to Region Outlines* check box in the General Preferences dialog box (Preferences command, File menu). If you remove a region (by right-clicking and selecting Delete Region from the pop-up context menu that appears), the numbering will be updated. After you have defined or edited your regions of interest using the Define Regions for Measurement command, MetaFluor will open the measurements graphs automatically.

Note: The pop-up context menu that appears when you right-click a region also contains commands that allow you to change the color of the region outline, shrink the region outline to fit a thresholded object, or copy the region outline to the Clipboard (and paste it into another image window).

WARNING:
If you move or remove a region while graphing the data, all data in the graphs will be erased. Exception: If you have selected the *Do Not Clear Graph When Regions Change* check box in the General Preferences dialog box (Preferences command, File menu), the graphs will not be cleared if you add or remove regions. Instead, the traces corresponding to those regions will disappear or be added to the graphs.

Note: If you move or redefine regions with the Define Regions for Measurement command while saving data to a measurements file, and want to log the new region information automatically (new coordinates, size, and thresholded area), you can do so by selecting the *Log Header After Editing Regions* check box in the Data Logging Preferences dialog box (Preferences command, File menu). This will *not* be the case when regions are moved with the Move All Regions command, however, because a large number of meaningless log entries would be generated during the movement and resizing procedures.

Define Regions for Measurement will save your measurement regions automatically to a file called "Last.rgn," that will be stored in your default regions directory. In this way, you will be able to use the most recent set of regions if you quit MetaFluor and then realize that you want to use those same regions when you restart the program.

TIP: To bring otherwise obscured graph windows to the "top" of your workspace display, use the Bring Graphs to Front command (Windows menu), or use its keyboard shortcut, [CTRL] + [G].

See Also:

Preferences

Configure Graphs

Clear Graphs

Move All Regions

Reference Images

Defining Regions for Measurement

To define or edit regions, use the procedure presented in the following table.
Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Step	Action
1	From the Graphs menu, choose Define Regions for Measurement. The Select Image for Defining Regions dialog box will appear.
2	If you are about to acquire images and want to perform measurements during your experiment, choose *Acquire Images* to acquire one set of images.
	It is important to acquire a pair of images now, so that you will know where to define regions of interest; otherwise the image display will be the last image in the video board's memory-- which could be its test pattern image.
3	From the *Image* list, select the image that you think is best suited for defining regions. Depending on the dye and other conditions, one image may be better than the others.
4	Choose *OK* to continue.
	OR
	Choose *Cancel* to cancel the Define Regions for Measurement command.
5	MetaFluor will temporarily close all image windows and dialog boxes except the image selected in Step 3. It will open the Region Toolbar and the Edit Regions dialog box.
6	Define the regions you want to measure using the Rectangular Region, Ellipse Region, Trace Region, or Auto-Trace Region Tool. You can move and resize the regions using the Locator Tool by holding down the [CTRL] key while you drag the region or its borders. You can move all regions simultaneously by holding down the [SHIFT] key while dragging any region. If necessary, you can choose *Clear Regions* to remove all of the defined regions from the image.
	OR
	Choose *Load* if you want to use regions that you have saved to disk. The Load Region File dialog box will appear. Select the desired *.rgn file. If necessary use the *Look In* list or Up One Level button to select the appropriate drive and folder. Then choose *Open.*
7	If you want to save the defined regions for later use, choose *Save.* The Save Region File dialog box will appear. Type a file name for the

region file in the *File Name* text box and choose *Save.*

Note: Define Regions for Measurement also automatically saves your last set of measurement regions to a file called "Last.rgn," that will be stored in your default regions directory.

8   If you want to print the image, complete with the region outlines (and region labels, if you selected the *Draw Labels Next to Region Outlines* check box in the General Preferences dialog box), choose *Print,* and follow the procedure for **configuring and printing** the image.

9   After you have finished editing or defining regions, choose *Done.*

MetaFluor will close the Region Toolbar and the Edit Regions dialog box, and restore the image windows and dialog boxes that were open prior to editing the regions. The measurements graphs will also be opened.

Printing an Image While Defining Regions

To print the image, use the procedure presented in the following table:

Step	Action
1	From the Edit Regions dialog box, choose *Print.* The Print with Region Outlines dialog box will appear.
2	In the *Image Title* text box, type a title for the image. This title will be printed at the top of the image.
3	Select a stamp color for the region outlines and labels *(Black* or *White)* from the *Stamp Color* option button group.
4	When you are ready, choose *Print.* The standard Windows Print dialog box will appear. Proceed as you would for printing any document in Windows.

Select Source Image for Defining Region - Dialog Box Options

Source Image

Selects the image to be used when defining the region of interest. Depending on the dye and other conditions, one image may be better than the others.

Acquire Images

Acquires one pair of images. You should use this command before defining the region, so that you will know where to place the region. Otherwise, the image displayed will be the last image in the video board's memory--which could be its test pattern image.

OK

Closes all image windows and dialog boxes except for the selected image and opens the Define Save Region dialog box so that you can define the region of interest.

Cancel

Cancels the Select Save Region command.

Edit Regions - Dialog Box Options

Load

Loads a region (*.rgn) file from disk. This option opens the Load Region File dialog box. Select the desired file. If necessary use the *Look In* list to select the appropriate drive and folder.

Save

Saves regions, currently defined on the image, to disk. This option opens the Save Region File dialog box. Type the desired file name for the .rgn file in the *File Name* text box. If necessary use the *Save In* list to select the location where you want to save the file.

Clear All

Clears all regions from the image.

Reset Colors

Resets the use of colors for region outlines back to the top of the order (i.e., to red) in the color menu. This is the same menu as is seen when you right-click a region and choose Change Color from the pop-up shortcut menu that appears.

Create Regions Around Objects

Automatically turns on thresholding for bright objects and creates regions around objects limited by your selections for *Min Size*, *Max Size*, and the *Max # of regions*.

Min Size

The total area of the smallest size object to threshold, measured in number of pixels.

Max Size

The total area of the largest size object to threshold, measured in number of pixels.

Max # of regions

The maximum number of regions to create in the image. The maximum value that you can enter for the *Max # of regions* is 255.

Create

Creates regions in the image according to the values that you specified for *Min Size*, *Max Size*, and the *Max # of regions*.

Print

Opens the Print with Region Outlines dialog box, from which you can configure and print the image with region outlines (and labels, if you selected the *Draw Labels Next to Region Outlines* check box in the General Preferences dialog box), superimposed on the image. If there are no regions of interest defined on the image, this command simply prints the image. Select the color to be used for printing the outlines using *Stamp Color*. Type the desired title for the printed image in the *Image Title* text box.

Regions Defined

Indicates the total number of defined regions in the active image window.

Active Region

Displays the number of the currently active region.

Average Intensity

Gives the average grayscale value for the currently active region.

Shortcuts!

Displays a message box explaining the [SHIFT] and [CTRL] key shortcuts for manipulating regions.

Done

Use this command button when you have finished editing regions. MetaFluor will close the Region Toolbar and the Edit Regions dialog box. It will also restore the image windows and dialog boxes that were open prior to editing regions. The measurements graphs will opened (you may need to choose the Show or Hide Graphs command from the Windows menu to make them visible).

Configure Graphs (Graphs Menu)

Specifies the region intensity and ratio data to be plotted, and configures the title, X and Y axis range, tick marks, labels, and line types, for up to five measurements graphs.

Use this command to configure the available options separately for each measurements graph. For each graph, you can configure the minimum and maximum grayscale or ratio values to be plotted on the Y-axis and the length of time on the X-axis. You can specify logarithmic scaling of the Y-axis, and you can specify that the Y-axis be inverted.

WARNING:
Because the Y-axis ranges for intensity and ratio data can differ so greatly, we strongly recommend that you configure separate graphs for intensity and ratio data.

This command does not open the graphs; they will be opened automatically after you have defined your measurement regions using the Define Regions for Measurement command. (You may still need to use the Show or Hide Graphs command in the Windows menu to make them visible.)

Note: At least one region must be defined on an image for data to be displayed in its corresponding measurements graph.

WARNING:
If you change the graph assignment *(Appears on Graph)* or line appearance *(Line Type)* for any data, all of the graphs will be erased.

See Also:

Define Regions for Measurement

Show or Hide Graphs

Configuring Region Data Measurements Graphs

To configure the measurements graphs, use the procedure presented in the following table:

Step	Action
1	From the Graphs menu, choose Configure Graphs. The Configure Graphs dialog box will appear.
2	For each data type in the Measured Value column *(Wavelength 1 Average Intensity,* etc.), select the graph to display it in, using the associated *Appears on Graph* drop-down list. (To omit a data type from graphing, select *None* from the *Appears on Graph* list.) **AND** Select the appearance of its data curve *(Solid, Wide, Dotted,* or *Dashed)* from the *Line Type* drop-down list. **WARNING:** Because the Y-axis ranges for intensity and ratio data can differ so greatly, we strongly recommend that you configure separate graphs for intensity and ratio data.
3	From the *Graph* drop-down list, select a graph for which you have assigned data in Step 2.
4	Select the *Show Graph* check box if you want to enable display the selected graph (depending on the state of the Show or Hide Graphs command in the Windows menu). If you want to configure the graph, continue to Step 5. Otherwise skip to Step 9. **OR** If you do not want to display the selected graph, clear the *Show Graph* check box. All options below the check box will become unavailable. Now skip to Step 9.
5	If necessary, type a more descriptive title for the selected graph in the *Title* text box under the *Graph* drop-down list.
6	Configure the *Y Axis* options by Typing an axis title in the *Title* text box of the *Y Axis* option group, Selecting the minimum and maximum grayscale or ratio values you want to plot, using the *Range* spin boxes, and Selecting the number of tick marks to display on the Y-axis, using the *Ticks* spin box. The *Tick Marks Every* status line will update to reflect the values selected in the *Range* and *Ticks* spin boxes.

7   If you want to use an inverted scale, such that a grayscale or ratio value of zero is at the top of the graph, select the *Invert Axis* check box.

   AND
   If you want to use a logarithmic scale, select the *Log Scaling* check box.

8   Configure the *X Axis* options by

   Selecting a time unit *(Seconds, Minutes,* or *Hours)* from the *Time* drop-down list,

   Selecting a range for the X-axis from the *Range* spin box (if you selected *Seconds* or *Minutes* as the *Time* unit, the *Range* value should be divisible by six), and

   Selecting the number of tick marks to display on the X-axis, using the *Ticks* spin box. The *Tick Marks Every* status line will update to reflect the values selected in the *Range* and *Ticks* spin boxes.

   Note: If you are using other graphs and want to use the same X-axis configuration, choose *Set All X Axes This Way.*

9   Repeat Steps 3 - 8 for any other graphs for which you have assigned data in Step 2.

10   When you are satisfied with your configurations, choose *OK.*

Configure Graphs - Dialog Box Options

Measured Value

Indicates which data will be assigned to the graph selected from the corresponding *Appears on Graph* drop-down list.

Appears on Graph

Selects a graph to which the associated data *(Wavelength 1 Average Intensity,* etc.) will be assigned. To omit a particular set of data from graphing, select *None.*

Line Type

Assigns a type of line *(Solid, Wide, Dotted,* or *Dashed)* to the data curve for the associated data *(Wavelength 1 Average Intensity,* etc.).

Graph

Selects a measurements graph to be configured. You can select from a total of 5 graphs and configure them as needed.

Title (Configure Graph Display options)

Assigns a title to the selected graph. The default title is *Graph 1.*

Show Graph

Enables display of the selected graph. (Whether the graph is actually displayed is also depends on the state of the Show or Hide Graphs command in the Windows menu).

Title (Y Axis options)

Assigns a title to the Y-axis of the selected graph. For example, you might use "Calcium" for a ratio graph from a fura-2 experiment.

Range… to… (Y Axis options)

Selects a minimum and maximum value for display on the Y-axis of the selected graph.

Ticks (Y Axis options)

Selects the number of tick marks to be displayed on the Y-axis of the selected graph.

Tick Marks Every (Y Axis options)

This status line will display the number of Y-axis units between each tick mark. This will update to reflect the values selected in the *Range* and *Ticks* spin boxes.

Invert Axis

Graphs the data with the higher values for intensity, ratio, or calibrated value (for example, pH or calcium concentration) indicated on the Y-axis towards the origin.

Log Scaling

Graphs the data using a logarithmic scale.

Time

Selects a time unit for the X-axis: *Seconds, Minutes,* or *Hours.*

Range (X Axis options)

Selects a range of time to be displayed along the X-axis. The minimum and maximum values will update automatically. For example, if you select a *Range* of *60* seconds, the graph will update to show an X-axis range of 60 to 120 seconds when the 61st second is reached in the experiment.

Ticks (X Axis options)

Selects the number of tick marks to be displayed on the X-axis of the selected graph.

Tick Marks Every (X Axis options)

This status line will display the number of time units between each tick mark. This will update to reflect the values selected in the *Range* and *Ticks* spin boxes.

Set All X Axes This Way

Configures all graphs to use the current set of *X Axis* option settings.

OK

Accepts the current configuration of the measurements graphs and closes the dialog box.

Cancel

Cancels any changes made to the configuration of the measurements graphs and closes the dialog box..

Clear Graphs (Graphs Menu)

Removes all graphed data from the measurements graphs.

Use this command to clear data from the graphs so that you can start new graphs.

The Clock is not reset when the Clear Graphs command is used. To clear the graphs and reset the Clock, use the **Zero Clock** command instead.

Shortcut: CTRL + C

See Also:

Zero Clock

Clearing MetaFluor Graphs

To clear the measurements graphs, use the following procedure:

Step	Action
1	Select the Run Experiment menu.
2	Choose Clear Graph. MetaFluor will clear both graphs. **Note:** You can use CTRL + C, the keyboard equivalent for the Clear Graph command at any point to clear the graphs.

Clear Measurement Regions and Graphs (Graphs Menu)

Clears measurement regions defined by the Define Regions for Measurement command, clears the measurement data from the graphs, and then closes the graphs.

Use this command when you have finished measuring data and want to clear and close the measurements graphs. Although the graphs are windows, you must use the Clear Measurement Regions and Graphs command to close them.

If you decide to reopen the graphs, you will need to use the **Define Regions for Measurement** command again. After you have defined new measurement regions, the measurements graphs will open again.

If you want simply to remove the graphs temporarily from the desktop, but do not want to have to use the Define Regions for Measurement command all over again, you should use the Show or Hide Graphs command (Windows menu), or use its keyboard shortcut, the [F8] function key.

If you want to clear the data from the measurements graphs without closing them, you should use the **Clear Graphs** command.

See Also:

Define Regions for Measurement

Show or Hide Graphs

Clear Graphs

Clearing Measurement Regions and Graphs

To clear measurement regions and close the measurements graphs, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Clear Measurement Regions and Graphs.
2	A message will appear, asking if you want to clear the measurement regions from the graphs and then close the graphs.
3	Choose *Yes.*

Utilities Menu

Play Movie from Disk (Utilities Menu)

This wizard builds an on-screen movie of a set of selected set of intensity (wavelength) or ratio images that have been stored on disk. If you wish, you can save the movie as a single .avi file.

Drop-in: MOVIE

The Play Movie from Disk wizard builds a movie using images that have been stored on disk. The movie can be played forward, backward, frame-by-frame, or in a loop.

Note: Before using this command, you must first install the **Movie** Drop-in using the *Configure Drop-ins/Toolbars* dialog box in the Meta Imaging Series Administrator.
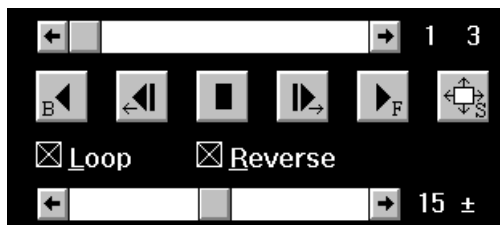
(Click each movie control in this illustration to learn more. Press [CTRL] + [TAB] to see the "hotspots.")

When you build a movie, you need to have enough memory to hold two sets of images: the original stack of ratio images and those used for the movie. A "small" movie requires less memory than a "large" movie (approximately 20 images is ideal for a movie). But that doesn't necessarily mean you must use fewer images when building a movie. There is another way to fit more images into memory with the use of the Zoom Tool: zoom the ratio stack to 50% before building the movie. The movie's size will be one-fourth the size of the original, and will require one-fourth the memory of a full-sized movie.

Note: This will affect only the amount of RAM used to play the movie. The amount of disk space used by a movie saved as an .avi file will be the same as that for a movie whose window size was not decreased.)

Shortcuts for Movie Controls:

[B]	Play movie backward.
<-	Play movie one frame backward.
[SPACE BAR]	Stop the movie.
->	Play movie one frame forward.
[F]	Play movie forward.
[S]	Maximize the movie.
[ESC]	Return the movie to standard

movie size.

Digital video information can consume large amounts of memory, particularly if the video frames are in 24-bit color. Because of this, you may want to use one of the compression formats if you save your movie as an .avi file. The .avi format has several standard compression formats:

Radius Inc. Cinepak: A 32-bit video codec (compression-decompression engine) that works best for compressing 24-bit color video images. This format provides greater compression, higher resolution, and faster playback than the Microsoft Video codec. You can specify your desired tradeoff between image quality and compression.

Intel Indeo Video Release 3.2: Another 32-bit video codec that works best for compressing 24-bit color video images. As with the Cinepak codec, you can specify your desired tradeoff between image quality and compression.

Microsoft Video 1: A 32-bit lossy compressor that works best with 8-bit and 16-bit images. You can specify separate settings for your desired tradeoffs between (1) compression and image quality, and (2) compression and temporal resolution.

Microsoft RLE: A 16-bit compressor that uses run-length encoding. Best for binary or 8-bit high-contrast images.

In addition to these codecs, two uncompressed formats are also available:

Intel Indeo Video Raw (16), and

Full Frames (Uncompressed).

Note: If you are saving the movie as an .avi file and want to play it back on a Macintosh or using the Apple QuickTime (TM) player, you must use the Cinepak codec so that the appropriate look-up table values are saved along with the .avi information.

For more information, click the following icons to view Microsoft's Web page on

» **Installing and Removing Codecs in Windows,** or

» **Troubleshooting Video Codecs in Windows 95.**

(Note: Be sure to enable use of "cookies.")

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Creating a Movie from a Set of Images

To build a movie from a set of intensity (wavelength) or ratio images, follow the directions described in the wizard's dialog box, as described in the following table:

Step	Action
1	From the Utilities menu, choose Play Movie from Disk. The Play Movie from Disk--Select Images dialog box will appear.
2	Follow the directions described in this first dialog box for selecting the first and last ratio images in the movie. Then choose *Next >>.*
3	As each of the wizard's dialog boxes appears, follow the directions that are described.
4	After you have followed all sets of instructions, an image window entitled "Movie" will appear on your desktop. You can choose *Play* to play through the images, or you can choose *Movie* to build a movie, which will be displayed in a separate movie Viewer window which has a control panel for playing the image frames, setting the playback speed, and maximizing the image window.
5	If you wish to save the movie as an .avi file, choose *Save As.* The Save as AVI Movie File wizard will appear. Follow the instructions for selecting a destination file, the "per frame" playback speed, and the compression mode.
6	When you have finished, choose *Close.*

Build INF File (Utilities Menu)

Creates a new .inf file from certain types of images.

This command can be used to create a new .inf file from MetaFluor images, Image-1/FL images, sequentially named files, multiple sequential files, a stack file, multiple stack files, RGB .tif files, and Bio-Rad confocal TCSM (*.pic and *.cmt) files.

For MetaFluor images, this command is useful for creating a new .inf file if the original .inf file was deleted. If you want to build an .inf file from MetaFluor images, you should select *MetaFluor Experiment* as the *Source Image Type.*

You can also use this command to create an .inf file for Image-1/FL images where Image-1/FL saved interlaced pair images (such that there is only one file per time point). If you want to build an .inf file from Image-1/FL images, you should select *Image-1/FL Experiment* as the *Source Image Type.*

MetaFluor does not need to build an .inf file for MetaGFP images; the MetaGFP .inf file can be read directly by MetaFluor. If you do want to build an .inf file from MetaGFP images, you should select *MetaFluor Experiment* as the *Source Image Type.*

If you have images with sequential names (not extensions) that were created in MetaMorph or some other software, you can use the Build INF File command to create an .inf file. The images will be renamed appropriately for experiment playback. You can specify the name of wavelengths so that every *n*th image corresponds to a different wavelength. If you have several sequential file series (such as Fluor01.tif, Fluor02.tif, Rhod01.tif, Rhod02.tif, FITC01.tif, FITC02.tif, etc.), MetaFluor will read these images, rename them, and generate an .inf file. Each sequence becomes a different wavelength in MetaFluor. Appropriate experiments will consist of sequential files named *Filename*XXX.tif (or any extension) where "XXX" is a sequence number (001, 002, etc.). The wavelength control will let you skip every *n*th image if the experiment has more than one wavelength. If you do want to build an .inf file from sequential images, you should select either *Sequential Files* or *Multiple Seq. Files* as the *Source Image Type.*

The Build INF File command has an option to obtain the time from the DOS timestamp on the image file. Or you can select the spacing in time between images. The DOS stamp is only accurate to within two seconds. If the images were saved far apart from each other (for example, every 30 seconds), this option can be useful. Otherwise, you should specify the time spacing that MetaFluor is to use.

The Build INF File command is useful for taking images from some external source (such as a confocal microscope) and converting them into a form that can be analyzed by MetaFluor.

Building an INF File for MetaFluor, MetaGFP, or Image-1/FL Images

To build an .inf file for MetaFluor, MetaGFP, or Image-1/FL images, use the following procedure:

Step	Action
1	From the Utilities menu, choose Build INF File. The Build INF File dialog box will appear.
2	From the *Source Image Type* list, select *MetaFluor Experiment* if you need to build an .inf file for MetaFluor of MetaGFP images. **OR** Select *Image-1/FL Experiment* if you are importing Image-1/FL files.
3	Choose *Select First Image* to specify the first image in the experiment. The First Image dialog box will appear. Select the icon for the first image file. If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder.
4	Choose *Select Last Image* to specify the last image in the experiment. The Last Image dialog box will appear. Select the icon for the desired image file.
5	If the image times can be determined from the image files, select *Determine from Files* from the *Image Times* group. **OR** Select *Equally Space* from the *Image Times* group. Then specify the amount of time using *Equally Space Images By*.
6	Choose *OK*. The Build INF File dialog box will close and the .inf file will be built for the MetaFluor or Image-1/FL images.

Building an INF File for Sequentially Named Images

To build an .inf file for sequential images, use the following procedure:

Step	Action
1	From the Utilities menu, choose Build INF File. The Build INF File dialog box will appear.
2	Select the type of images that you are using to build the .inf file from the *Source Image Type* list (*Sequential Files* or *Multiple Seq. Files*).
3	Choose *Select First Image* to specify the first image in the experiment. The First Image dialog box will appear. Select the icon for the first image file. If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder.
4	Choose *Select Last Image* to specify the last image in the experiment. The Last Image dialog box will appear. Select the icon for the desired image file.
5	If you selected *Sequential Files* as the selected *Source Image Type* in Step 2, select the number of wavelengths using *Number of Wavelengths*. **OR** If you selected *Multiple Seq. Files* in Step 2, continue to Step 6.
6	Select the number of digits in the sequence number using *Number of Sequence Digits*.
7	If you selected *Multiple Seq. Files* in Step 2, type the base names for the second and third wavelengths in the *2* and *3* text boxes of *Base Names for Wavelengths 2 – 4*. **OR** If you selected *Sequential Files* in Step 2, skip to Step 9.
8	If the image times can be determined from the image files, select *Determine from Files* from the *Image Times* group. **OR** Select *Equally Space* from the *Image Times* group. Then specify the amount of time using *Equally Space Images By*.
9	Choose *OK*. The Build INF File dialog box will close and the .inf file will be built for the sequential images.

Building an INF File for Image Stacks

To build an .inf file for stack images, use the following procedure:

Step	Action
1	From the Utilities menu, choose Build INF File. The Build INF File dialog box will appear.
2	Select the type of images that you are using to build the .inf file from the *Source Image Type* list (*Stack File* or *Multiple Stack Files*).
3	Choose *Select Stack Image*. Then select the icon for the .stk file. (If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder.) Then choose *Open*.
4	If you selected *Stack File* in Step 2, select the number of wavelengths using *Number of Wavelengths*. Then skip to Step 6. **OR** If you selected *Multiple Stack Files*, skip to Step 5.
5	If you selected *Multiple Stack Files* in Step 2, type the base names for the second and third wavelengths in the *2* and *3* text boxes of *Base Names for Wavelengths 2 – 4*. **OR** If you selected *Stack File* in Step 2, skip to Step 6.
6	In the *Equally Space Images By* option, specify the amount of time between images.
7	Choose *OK*. The Build INF File dialog box will close and the .inf file will be built for the stack images.

Building an INF File for RGB TIFF Images

To build an .inf file for RGB TIFF images, use the following procedure:

Step	Action
1	From the Utilities menu, choose Build INF File. The Build INF File dialog box will appear.
2	From the Source Image Type list, select Sequential RGB TIFF Files.
3	Choose *Select First Image* to specify the first image in the experiment. The First Image dialog box will appear. Select the icon for the first image file. (If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder.) Then choose *Open*.
4	Choose *Select Last Image* to specify the last image in the experiment. The Last Image dialog box will appear. Select the icon for the desired image file. Then choose *Open*.
5	From the *Number of Wavelengths* spin box, select the number of wavelengths in the experiment.
6	If the image times can be determined from the image files, select *Determine from Files* from the *Image Times* group. **OR** Select *Equally Space* from the *Image Times* group. Then specify the amount of time using *Equally Space Images By*.
7	Choose *OK*.
8	A message will appear, asking you to verify the .inf file creation. Choose *OK*. The Destination Name dialog box will appear.
9	Type a name for the .inf file in the *File Name* text box. Then choose *Save*. The Sequential RGB TIFF Files dialog box will appear.
10	Select which color components are to be loaded into which wavelength image by selecting a color component from the *Load Wavelength 1 With*, *Load Wavelength 2 With*, and *Load Wavelength 3 With* lists.
11	Choose *OK*. The Build INF dialog box will close, and the .inf file will be built for the RGB .tif files.

Building an INF File for Bio-Rad Confocal TCSM Images

To build an .inf file for Bio-Rad confocal TCSM images, use the following procedure:

Step	Action
1	From the Utilities menu, choose Build INF File. The Build INF File dialog box will appear.
2	From the *Source Image Type* list, select *Biorad TCSM Files.*
3	Choose *Select TCSM PIC.* The First Image dialog box will appear. Select the icon for the desired .pic file. (If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder.) Then choose *Open.*
4	With the *Number of Wavelengths* spin box, select *1* if you are importing single images, or select *2* if you are importing side-by-side paired images.
5	If you selected *2* in Step 4 and want the left side of the .pic to be the Wavelength 1 image, select *[Wavelength 1] : [Wavelength 2]* from the *Image Position* option button group. If you want the Wavelength 1 image to be on the right, select *[Wavelength 2] : [Wavelength 1].*
6	Choose *OK.*

Build INF File - Dialog Box Options

Source Image Type

Specifies the type of images that you are using to build an .inf file. Select:

MetaFluor Experiment if you want to rebuild an .inf file for a MetaFluor or MetaGFP experiment after the original .inf file was deleted.

Image-1/FL Experiment if you want to build an .inf file for interlaced image pairs from an Image-1/FL experiment so that you can open the Image-1/FL experiment in MetaFluor.

Sequential Files if you want to build an .inf file for images that were created by MetaMorph or some other type of software that saves images with sequential names sharing the same extension.

Multiple Seq. Files if you have several series of sequential files. MetaFluor will read the images and rename before creating an .inf file. Select *Stack File* if the images are in a .stk or .spe file.

Stack File if the images are stored as planes in a single stack file.

Multiple Stack Files if the images for each wavelength are stored in separate stack files. Stack 1 will correspond to Wavelength 1, Stack 2 will correspond to Wavelength 2, etc. If you select this image file type, all stack files must be located in the same folder. The .inf file that will be created, as well as all of the sequentially numbered TIFF files (for example, Image1.001, Image2.001, etc.) will be stored in the same folder.

Sequential RGB TIFF Files if the images are stored in RGB .tif files. MetaFluor reads the RGB images and then resaves the image data into separate files which are named according to MetaFluor conventions *(Filename*1.001, *Filename*2.001, etc.). If the RGB TIFF images are from a Zeiss confocal microscope, an experiment notebook file containing the Zeiss annotation information for each image will be created and saved. If you select *Sequential RGB TIFF Files,* the Sequential RGB TIFF Files dialog box will appear. This window allows you to specify which color components are to be loaded into which wavelength by selecting *Red Component, Green Component,* or *Blue Component* from the *Load Wavelength 1, Load Wavelength 2,* and *Load Wavelength 3* drop-down lists.

BioRad TCSM Files if the images are stored as Bio-Rad TCSM .pic files. After MetaFluor performs the conversion, you will have the .inf file, a Notebook (*.txt) file, two .tif files containing the background images, and a series of image files *(Filename*.001, *Filename*.002, and so on). These will all be stored in the same directory as the original .pic file. **Note:** You must have **both** the .pic file and the .cmt text file available for the conversion, and they must be in the same directory. The .cmt text file contains the annotation text that specifies the experimental event marks and image timestamps. These will be displayed in the new Notebook file.

Select First Image

Specifies the first image of the experiment. When you select *Multiple Seq. Files,* MetaFluor assumes that each of the series of sequences has the same sequence numbering.

Select Stack Image

When *Stack File* is selected as the *Source Image Type,* this command button specifies the stack file containing the images from which the .inf and ratio images will be created.

When you select *Multiple Stack Files* as the *Source Image Type,* this command button selects the stack file for the first Wavelength image.

Select TCSM PIC

Specifies the Bio-Rad confocal TCSM .pic image file to be used.

Select Last Image

Specifies the last image of the experiment. When *Multiple Seq. Files* is selected, MetaFluor assumes that each of the series of sequences has the same sequence numbering.

Number of Wavelengths

Specifies the number of wavelengths for the experiment. When you specify more than one wavelength, MetaFluor will skip every *n* images to accommodate the additional wavelengths.

Image Position

Selects an arrangement for the confocal image pair, with the Wavelength 1 image on the left or on the right. This option appears only if you have selected *Biorad TCSM Files* as the *Source Image Type.*

Base Names for Wavelengths 2 - 4

This option appears only if you have selected *Multiple Seq. Files* or *Multiple Stack Files* as the *Source Image Type.* When you want to build an .inf file from a series of sequential files that have different base names, such as Fluor01.tif, Fluor02.tif, Rhod01.tif, Rhod02.tif, FITC01.tif, FITC02.tif, etc., you will need to specify the base names for the second, third, and fourth series. Use this option to specify the base names for the other wavelength series.

Image Times

Select *Determine from Files* if the times can be determined from the image files. Otherwise select *Equally Space* and set the time using *Equally Space Images By.*

Equally Space Images By

Use this option to specify the amount of time between images if you selected *Equally Space* from the *Image Times* group. For some source types, you must equally space images because the times cannot be determined from the images. You must use this mode if you select *Multiple Stack Files* as the *Source Image Type.*

Description

Provides a description of the type of experiment that has the selected *Source Image Type.*

OK

Builds the .inf file.

Cancel

Cancels the command.

Delete Images (Utilities Menu)

Selectively deletes images from a stored experiment.

Use this command to delete unwanted images from an experiment. After you have marked the images you want to delete, MetaFluor will delete them and reopen the experiment with the remaining images. The experiment must be open before you can delete images from it.

Deleting Images from a Stored Experiment

To delete images from a stored experiment, use the following procedure:

Step	Action
1	Open the experiment from which you want to delete images, using the **Open Experiment** command.
2	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel will appear. Leave this open in the MetaFluor window.
3	From the Utilities menu, choose Delete Images. The Delete Images dialog box will appear.
4	Use the *Frame* slider in the Experiment Control Panel to step through your experiment, frame by frame. The highlighted frame in the Delete Images dialog box's list box will update as you do this.
5	When you find a frame that you want to delete, double-click its image number in the list box. This will mark the image with an asterisk (*). Only marked images will be deleted.
	If you don't want to delete an image that you have marked, double-click its image number again to remove the asterisk.
6	When you have marked all unwanted frames, choose *Delete.* A message will appear, asking you to confirm your selection.
	If you want to delete the images listed in the message, choose *OK.* Otherwise choose *Cancel* to cancel the Delete Image command.
7	MetaFluor will then delete the images and display another confirmation message.
	Choose *OK,* and the experiment will be reopened, minus the unwanted images.

Delete Images - Dialog Box Options

Index/Time List Box

Displays a list of all of the images in the experiment and their times. Those marked with an asterisk (*) will be deleted when you choose *Delete.* Double-clicking an image number and time toggles the asterisk on and off.

Delete

Displays a confirmation dialog box listing the images you have selected to delete. When you confirm this message by choosing *OK,* MetaFluor will delete the images marked with an asterisk (*).

Cancel

Cancels the command.

Import N-Dimensional Imaging Sequence

This wizard imports a sequence of images acquired with a "multi-dimensional" protocol. An example might be a Z-series or a series of wavelength images (first dimension) that were acquired at a number of stage positions (second dimension) at a number of timepoints (third dimension).

Drop-in: IMPORTND

Use this command to import sequential images into MetaFluor that were acquired using a "multi-dimensional" acquisition sequence. Such images may have been generated by journals in the MetaMorph Imaging System. An example of such a sequence is the case where a Z-series has been acquired at each location in a multi-well plate. This acquisition sequence is then repeated at each of a number of timepoints.

Before using this command, you must install its drop-in, IMPORTND, using the MetaMorph Meta Imaging Series Administrator.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Importing an N-Dimensional Imaging Sequence

To import an *N*-dimensional imaging sequence, use the following procedure:

Step	Action
1	From the Utilities menu, choose Import *N*-Dimensional Imaging Sequence. The Import *N*-Dimensional Imaging Sequence dialog box will appear.
2	Choose *First Image.* The Select First Image dialog box will appear.
3	Select the icon representing the first image in the sequence, and choose *Open* to return to the Import *N*-Dimensional Imaging Sequence dialog box.
4	Use *Number of Sequence Digits in File Name* to specify the number of spaces in the file name that are taken up by the sequence number.
5	Use the other spin boxes to specify the "dimensions" of the image sequence, as described in the dialog box.
6	When you have finished, choose *Next* and follow the directions described in the second dialog box for specifying the stage position, Z-distance, number of wavelengths, and the starting wavelength. Then choose *Next.*
7	In the third dialog box that appears, choose *Select File,* and type a file name for the imported files' new .inf file in the *File Name* text box of the INF Experiment Name dialog box that appears. Then choose *Save* to return to the Import *N*-Dimensional Imaging Sequence dialog box.
8	Choose *OK.* The final page of the Import *N*-Dimensional Imaging Sequence wizard will appear.
9	When you are ready, choose *Begin.* The *Status* line will indicate the progress of the import procedure.
10	When you have finished, choose *Close.*

Save as 8-Bit Image (Utilities Menu)

Saves a 16-bit wavelength image as an 8-bit .tif file.

Drop-in: SAVE8BIT

Use this command when you want to convert a 16-bit wavelength image to 8-bit format. This will allow you to export the image for use by programs that require an 8-bit format, such as a desktop publishing or word processing program. The grayscale values in the image will be scaled automatically.

Before using this command, you must install its drop-in, SAVE8BIT, using the MetaMorph Meta Imaging Series Administrator.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Scale 16-Bit Image

Saving as an 8-Bit Image

To save a 16-bit wavelength image in an 8-bit .tif image file, use the following procedure.

(Note: You must first open a stored experiment that has its wavelength images saved as 16-bit images.)

Step	Action
1	With the pointer, click the title bar of the wavelength image that you want to save, so that its window becomes the active window.
2	From the Utilities menu, choose Save as 8-Bit Image. The Save as 8-Bit Image dialog box will appear.
3	In the *File Name* text box, type the name under which you want to save the image. If necessary, use the *Save In* list or Up One Level icon button to specify a different drive or folder in which to save the image file.
4	Choose *Save.* The image file will be saved with the extension ".tif" appended to the file name, and the dialog box will close automatically.

Save as 8-Bit Image - Dialog Box Options

File Name

Lists the name of the currently selected file.

Files of Type

Determines the file format of the files displayed in the *File Name* list. The default format is **.TIF.*

Save In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level icon button to go up one level in the directory structure.

Save

Saves the 16-bit wavelength image as an 8-bit .tif file.

Cancel

Cancels the command.

Spot Measurements (Utilities Menu)

Displays the grayscale intensities and ratio values from all wavelength and ratio images for the pixel selected by the pointer when you click over a location in one of the images. If the images have been calibrated, the calibrated value will also be displayed.

Use this command to determine typical intensity, ratio, and calibrated values. In addition to displaying the intensity, ratio, and calibrated values, this command also displays the location (X and Y coordinates) of the measurement. Although the intensity values are displayed in MetaFluor's Status Bar, the ratio and calibrated values are not. Therefore, you should use the Spot Measurements command when you need to check these values quickly. This command is useful, for example, in determining the darkest and brightest values in the images when setting up fixed scaling for 16-bit images.

This command is available whenever you display images in an image window on your computer screen. If you are displaying your images on an external video monitor, you can use this command on the image displayed for the Define Regions for Measurement command (Graphs menu).

See Also:

Define Regions for Measurement

Making Spot Measurements

To make spot measurements, use the following procedure:

Step	Action
1	From the Utilities menu, choose Spot Measurements. The Spot Measurements dialog box will appear.
2	Position the pointer over one of the wavelength or ratio images at the location you want to measure, and press the mouse button.
3	MetaFluor will display the coordinates for the measurements and the intensity and calculated measurements in the Spot Measurements dialog box.
4	Repeat Step 2 for each location you want to measure.
5	Choose *Close* when you have finished.

Spot Measurements - Dialog Box Options

Measurement At (XY:)

Displays the X and Y coordinates of the current measurement.

Wavelength *N* (W#)

Displays the gray level intensities at the selected location for the pertinent wavelength image.

Ratio *N* (R#)

Displays the ratio value at the selected location for the pertinent ratio image.

Calibrate *N* (C#)

Displays the calibrated value at the selected location. The calibrated value will be displayed only if the calibration has been configured.

More >>

Expands the dialog box. When the dialog box is expanded, the titles of the parameters will be given in full. When you choose *Less* << to condense the dialog box, an abbreviated form of the parameter titles (shown in parentheses in the preceding four descriptions) will be displayed.

Less <<

Condenses the dialog box (see preceding description for the *More* >> button).

Close

Closes the dialog box.

Configure Intensifier Gain Control (Utilities Menu)

Configures the control of the intensifier CCD camera settings when using computer-controlled gain.

Drop-in: ICCD

Use this command before using the Set Intensifier Gain command and the Set Camera Level and Gain command with an intensified CCD camera (and the PI Video ICCD Settings command, if you are using this camera). Configure Intensifier Gain Control allows you to specify the camera model, serial port, and baud rate. It also allows you to select whether the camera is controlled by the computer or by the front-panel knobs on the intensifier. The camera must be controlled by the computer to use the intensifier gain commands in MetaFluor.

You must use this command before using the Set Intensifier Gain, the Set Camera Level and Gain, and PI Video ICCD Settings commands. These three commands will be unavailable until you do so.

Before using this command, you must install its drop-in, ICCD, using the MetaMorph Meta Imaging Series Administrator.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Set Intensifier Gain

Set Camera Level and Gain

PI Video ICCD Settings

Configuring the Intensifier Gain Control

To configure the intensifier gain control for use with an intensified CCD camera, use the following procedure:

Step	Action
1	From the Utilities menu, choose Configure Intensifier Gain Control. The Configure Intensifier Gain Control dialog box will appear.
2	Select your camera's model name from the *Intensifier Model* drop-down list.
3	Select the serial port used to connect the camera from the *Serial Port* drop-down list.
4	Select the appropriate baud rate for the connection from the *Baud* drop-down list.
5	To control the intensified CCD camera using the other intensified gain control commands in MetaFluor, select *Computer* from the *Camera Control* group. **Note:** If your camera requires manual control for some features on the camera, select *Manual* before performing those operations.
6	Choose *OK.*

Configure Intensifier Gain Control - Dialog Box Options

Intensifier Model

Specifies the intensified CCD camera model name.

Serial Port

Specifies the serial port used to connect the camera to the computer.

Baud

Specifies the baud rate used for the connection between the camera and the computer.

Camera Control

Switches between manual control and computer control of the camera. Use *Manual* when you want to change settings on the camera that can only be accessed on the camera when it is not controlled by the computer. Use *Computer* when you want to control the camera from MetaFluor.

OK

Configures the intensifier gain control options.

Cancel

Cancels the command.

Set Intensifier Gain (Utilities Menu)

Allows the user to set the intensifier gain when using an intensified CCD camera.

Drop-in: ICCD

You can use this command to set the intensifier gain interactively during acquisition. The same gain is used for all wavelengths that are acquired. You can change the gain setting by using a slider. Once you have changed the slider's value, the camera will use that value for all wavelengths acquired, starting with the next acquisition.

Alternatively, you can select intensifier gain settings for each wavelength prior to acquisition and then tell MetaFluor to switch the intensifier automatically to the gain setting selected for a wavelength right before acquiring that wavelength. When using this alternate method, you may want to specify a delay after the gain is set so that there is enough time before the acquisition for the gain to "lock in" to the correct setting.

Before using this command, you must install its drop-in, ICCD, using the MetaMorph Meta Imaging Series Administrator. You must also configure the intensifier using the Configure Intensifier Gain Control command and specify that the camera is controlled by the computer.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Configure Intensifier Gain Control

Set Camera Level and Gain

PI Video ICCD Settings

Setting the Intensifier Gain

To set the intensifier gain, use the following procedure:

Step	Action
1	From the Utilities menu, choose Set Intensifier Gain. The Set Intensifier Gain dialog box will appear.
2	If you want to set the gain values for each wavelength prior to acquisition, select the *Set Intensifier Gain Before Acquiring Wavelengths* check box. **OR** If you want to set the gain during acquisition, clear the *Set Intensifier Gain Before Acquiring Wavelengths* check box and skip to Step 5.
3	Select the *Set Wavelength 1 To* check box if you want the gain for Wavelength 1 to be changed automatically during acquisition. Then type the desired gain value in its text box. If you want a delay to occur after the gain has been changed, select the desired length using *After Setting Gain, Delay for… ms.*
4	Repeat Step 3 for any other wavelengths. Then skip to Step 6.
5	If you want to set the gain interactively, select the desired gain from the *Intensifier Gain* slider. You can choose *Use Current* to set that gain for the corresponding wavelength.
6	Begin your experiment. If the gain is being automatically switched by MetaFluor, you will see the current gain value change in the *Intensifier Gain* slider as the various wavelengths are acquired. If you are interactively changing the gain yourself, you can change the value selected in *Intensifier Gain* slider at any time.
7	Choose *Close* when you have finished.

Set Intensifier Gain - Dialog Box Options

Intensifier Gain

Allows you to change the intensifier gain interactively for all wavelengths during acquisition if *Set Intensifier Gain Before Acquiring Wavelengths* is disabled.

Set Intensifier Gain Before Acquiring Wavelengths

When selected, this option instructs MetaFluor to set the intensifier gain for each enabled wavelength to the specified value. The *Set Wavelength N To* options will also be unavailable when this option is disabled.

Set Wavelength 1 To

Selecting this option's check box instructs MetaFluor to set the intensifier gain automatically to the specified value before Wavelength 1 is acquired. Type the desired gain value in the text box. Or choose *Use Current,* and the value currently displayed in the *Intensifier Gain* slider will appear in this option's text box. This option is available only if *Set Intensifier Gain Before Acquiring Wavelengths* has also been selected.

Set Wavelength 2 To

Selecting this option's check box instructs MetaFluor to set the intensifier gain automatically to the specified value before Wavelength 2 is acquired. Type the desired gain value in the text box. Or choose *Use Current,* and the value currently displayed in the *Intensifier Gain* slider will appear in this option's text box. This option is available only if *Set Intensifier Gain Before Acquiring Wavelengths* has also been selected.

Set Wavelength 3 To

Selecting this option's check box instructs MetaFluor to set the intensifier gain automatically to the specified value before Wavelength 3 is acquired. Type the desired gain value in the text box. Or choose *Use Current,* and the value currently displayed in the *Intensifier Gain* slider will appear in this option's text box. This option is available only if *Set Intensifier Gain Before Acquiring Wavelengths* has also been selected.

Set Wavelength 4 To

Selecting this option's check box instructs MetaFluor to set the intensifier gain automatically to the specified value before Wavelength 4 is acquired. Type the desired gain value in the text box. Or choose *Use Current,* and the value currently displayed in the *Intensifier Gain* slider will appear in this option's text box. This option is available only if *Set Intensifier Gain Before Acquiring Wavelengths* has also been selected.

Set Wavelength 5 To

Selecting this option's check box instructs MetaFluor to set the intensifier gain automatically to the specified value before Wavelength 5 is acquired. Type the desired gain value in the text box. Or choose *Use Current,* and the value currently displayed in the *Intensifier Gain* slider will appear in this option's text box. This option is available only if *Set Intensifier Gain Before Acquiring Wavelengths* has also been selected.

Use Current

Displays the value currently displayed in the *Intensifier Gain* slider in the gain text box for the associated wavelength.

After Setting Gain, Delay for… ms

Specifies the length of the delay to occur before the next acquisition once the gain is changed.

Close

Closes the dialog box.

Set Camera Level and Gain (Utilities Menu)

Sets the intensified CCD camera's black level and video gain.

Drop-in: ICCD

Use this command to change the ICCD camera's black level and video gain. The control of the black level and video gain depends on the particular camera used. For some cameras, the black level and video gain can be set only by using the computer after control of the camera has been turned over the computer. In some cases, you may be allowed to change the black level and video gain either manually or with this command. However, some cameras do not support this command, and you must change the black level and video gain manually.

Before using this command, you must install its drop-in, ICCD, using the MetaMorph Meta Imaging Series Administrator. You must also configure the intensifier using the Configure Intensifier Gain Control command and specify that the camera is controlled by the computer.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Configure Intensifier Gain Control

Set Intensifier Gain

PI Video ICCD Settings

Setting the Camera Level and Gain

To set the ICCD camera black level and gain, use the following procedure:

Step	Action
1	From the Utilities menu, choose Set Camera Level and Gain. The Set CCD Level and Gain dialog box will appear.
2	Select the desired black level value from the *Black Level* slider.
3	Select the desired video gain value from the *Video Gain* slider.
4	Choose *Close.*

Set Camera Level and Gain - Dialog Box Options

Black Level

Specifies the current black level value used by the intensified CCD camera. For some cameras, the black level and video gain can be set only by using the computer after control of the camera has been turned over the computer. In some cases, you may be allowed to change the black level and video gain either manually or with this command. However, some cameras do not support this command and you must change the black level and video gain manually.

Video Gain

Specifies the current video used by the intensified CCD camera. For some cameras, the black level and video gain can be set only by using the computer after control of the camera has been turned over the computer. In some cases, you may be allowed to change the black level and video gain either manually or with this command. However, some cameras do not support this command and you must change the black level and video gain manually.

Close

Closes the dialog box.

PI Video ICCD Settings (Utilities Menu)

Changes the ICCD operating temperature and resets the intensifier if it shuts off due to overload. If you configured the system for direct gain control from the computer, additional setting controls that appear on the controller front panel will also be available.

Drop-in: ICCD

Use this command to reset the intensifier if it shuts itself down due to overload. You can also use the PI Video ICCD Settings command to specify an operating temperature for the cooled ICCD (the default setting is -10 degrees Centigrade).

If you specified direct control of the ICCD by the computer with the Configure Intensifier Gain Control command, a dozen other setting controls will appear in the PI Video ICCD Settings dialog box. For the most part, these correspond to controls that appear on the ICCD controller box, and include such options as enabling or disabling Auto-Black Level, Automatic Gain Control, responsiveness to external triggers, Micro-Channel Plate (MCP) protection circuitry, and the like.

Before using this command, you must install its drop-in, ICCD, using the MetaMorph Meta Imaging Series Administrator. You must also configure the intensifier using the Configure Intensifier Gain Control command.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Configure Intensifier Gain Control

Set Intensifier Gain

Set Camera Level and Gain

Specifying the PI Video ICCD Settings

To specify the PI video ICCD settings, use the following procedure:

Step	Action
1	Follow the directions for **configuring the intensifier gain,** selecting one of the PI Video ICCD entries from the *Intensifier Model* drop-down list box.
2	From the Utilities menu, select PI Video ICCD Settings. The PI Video ICCD Settings dialog box will appear.
3	If you want to change the operating temperature of the ICCD, use the *Temperature Set Point* spin box to specify the new temperature. The default setting is -10 degrees C.
4	If the intensifier has shut down due to overload, choose *Reset Intensifier If It Shut Off Due to Overload.* (Be sure all input to the camera is off!)
5	If you selected control of the ICCD by the control box *(PI Video ICCD - Control Box)* when you used the Configure Intensifier Gain Control command, you have finished. Now skip to Step 9.
	OR
	If you selected direct control of the ICCD by the computer *(PI Video ICCD - Direct)* when you used the Configure Intensifier gain Control command, you will see some additional options in the PI Video ICCD Settings dialog box. Continue to Step 6.
6	Depending on your experimental conditions, select or clear the *Advanced Settings* check boxes as necessary.
	Note: If you are performing quantitative densitometric or ratiometric analysis of your images, you should leave *Enable Automatic Gain Control* deselected.
7	If you are using an integrating ICCD, you can specify the number of video frames to be integrated by using *Frames to Integrate.*
8	If you need to reset *Advanced Settings* or *Frames to Integrate* to the default values, choose *Reset to Defaults.*
9	Choose *Close* to close the dialog box.

PI Video ICCD Settings - Dialog Box Options

Temperature Set Point

Specifies the operating temperature of the cooled ICCD. The default setting is *-10* degrees C.

Reset Intensifier If It Shut Off Due to Overload

If your ICCD becomes saturated due to an overload of input, it will shut down as a protective measure. When this happens, you can reset it by choosing this command button.

Advanced Settings

These check boxes can be selected or cleared independently, thereby emulating the controls on the ICCD controller box. These options will only be displayed if you selected *PI Video ICCD - Direct* as the *Intensifier Model* in the Configure Intensifier Gain Control dialog box. These options include the following:

Enable Micro-Channel Plate Protection Circuitry (default = enabled)
Enable Gamma of 0.45 (default = disabled)
Enable Automatic Gain Control (default = disabled)
Enable Continuous (CW) Intensifier Mode (default = enabled)
Positive Polarity EXT Trigger (default = enabled)
Not EXT Trigger Enabled (default = enabled)
Enable Odd Field for Trigger and Integration (default = enabled)
Enable Any Field for Trigger and Integration (default = enabled)
Turn Off Auto-Black Level (default = enabled)
INVERT Valid Polarity (default = enabled)

Frames to Integrate

If you are using an integrating ICCD, this option specifies the number of video frames to be integrated. A setting of *0* specifies no integration.

Reset to Defaults

Resets the *Advanced Settings* check boxes and *Frames to Integrate* spin box to their default settings.

Close

Closes the dialog box.

Convert PI MultiViewer Images (Utilities Menu)

Converts "split-screen" images from a single-wavelength experiment, such as those acquired with a Princeton Instruments MultiViewer, into separate single-frame images that can be stored as a dual-wavelength experiment.

Drop-in: PIMVIEW

Use this command to create single-frame images from paired "split-screen" images, such as those captured with the PI MultiViewer. Convert PI MultiViewer Images functions as a "wizard," presenting a series of four dialog boxes that walk you through the processes of selecting source images, defining the location of the split between the paired half-frame images, aligning the two images with each other, and executing the conversion. An experiment information (*.inf) file will be created automatically, using the name that you specify. The converted images can then be "played back" and analyzed as with any dual-wavelength experiment whose images were acquired with a conventional camera.

The Convert PI MultiViewer Images drop-in command is available only when there is no experiment currently open. If you are currently running an experiment or playing one back, you will need to close the experiment first before using this command.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Converting PI MultiViewer Images

To convert "split-screen" images into separate single-frame images, use the following procedure:

Step	Action
1	From the Utilities menu, choose Convert PI MultiViewer Images. The Convert PI MultiViewer Experiment dialog box will appear.
	This first page presented by the Convert PI MultiViewer Images wizard is used for selecting the source images and defining the orientation of the split between the paired images.
2	Choose *Select.* The Select Information File (INF File) dialog box will appear.
	AND
	Select the .inf file corresponding to the experiment whose images you want to convert. If necessary, use the *Look In* drop-down list box or Up One Level icon button to locate the correct drive and folder. Then choose *Open.*
3	From the *How Does the PI MultiViewer Split the Image* group, select the orientation of the split between the paired source images: *Vertically (Wave 1 Above Wave 2)* or *Horizontally (Wave 1 to the Left of Wave 2).*
4	Choose *OK.* The Convert PI MultiViewer Experiment dialog box will close, and the Define Split in Image dialog box will appear. This second page in the wizard is used for specifying the precise location of the split line between the two paired images.
5	Use the *Select an Image from the Experiment* slider to locate a suitable frame from among the experimental source images.
6	If you want to adjust the contrast of the image, you can either have MetaFluor do so automatically by selecting the *Use Automatic Scaling* check box, so that a check mark appears in it.
	Or you can select the scaling manually by clearing the *Use Automatic Scaling* check box and adjusting the intensity scaling limits with the *Low* and *High* sliders.
7	Now you can adjust the location of the split line between the two paired images. Drag the red split line until it is located as precisely as possible between the two half-images.
8	Choose *OK.* The Define Split in Image dialog box will close, and the Align Top and Bottom

dialog box or the Align Left and Right dialog box will appear, depending on whether you are using a vertical or horizontal split.

This third page in the wizard is used for adjusting the alignment between the two half-images as precisely as possible so as to obtain meaningful ratio images after the source images are converted.

9   From the *View Alignment Image As* group, select the method you want to use to view how well the superimposed half-images are lined up. Select:

Subtraction to use an image that subtracts the grayscale values of the second image from those the first, pixel by pixel,

Average to use an image that takes an average of the grayscale values of corresponding pixels in the two images,

Color to use an image in which the first half-image is rendered in red and the second is rendered in green, or

Ratio to use an image constructed by "ratioing" the pixel values in first image by those of the second image.

10   If necessary, use the *Select an Image from the Experiment* slider to locate a suitable frame from among the experimental source images.

11   As before, if you want to adjust the contrast of the alignment image, you can either have MetaFluor do so automatically by selecting the *Use Automatic Scaling* check box, so that a check mark appears in it.

Or you can select the scaling manually by clearing the *Use Automatic Scaling* check box and adjusting the intensity scaling limits with the *Low* and *High* sliders.

12   Now you can adjust the alignment of the two images. Use the *Left, Right, Up,* and *Down* buttons to shift the second (bottom, or right) image relative to the first (top, or left). The offset between the two images, relative to their original alignment, will be indicated by the *Current Offset* status line.

13   When you have finished, choose *OK.* The Align Top and Bottom (or Align Left and Right) dialog box will close, and the Convert MultiViewer Experiment dialog box will appear.

This last page of the wizard is used for carrying out the conversion process.

14   Choose *Select.* The New Experiment File (INF File) dialog box will appear.

       AND

       Type a name for the .inf file for the newly

converted images in the *File Name* text box. .
If necessary, use the *Save In* drop-down list
box or Up One Level icon button to locate the
desired drive and folder. Then choose *Save.*

15 When you are ready, choose *Begin.*

A progress meter will appear at the bottom of
the dialog box, indicating the progress of the
conversion process, and the names of the
newly created images will be displayed in a
status line. When the process is complete, the
dialog box will close automatically.

Convert PI MultiViewer Experiment - Dialog Box Options

Select

Opens the Select Information File (INF File) dialog box, from which you will select the .inf file for the original MultiViewer experiment whose images you want to convert.

How Does the PI MultiViewer Split the Image

Selects the orientation of the split between the paired half-images in the MultiViewer image frames. If the images are paired one above the other, you should select *Vertically.* If the images are paired side by side, you should select *Horizontally.*

OK

Accepts the dialog box settings, closes the Convert PI MultiViewer Experiment dialog box, and opens the Define Split in Image dialog box.

Cancel

Cancels the Convert PI MultiViewer Experiment command.

Convert MultiViewer Experiment - Dialog Box Options

Select

Opens the New Experiment File (INF File) dialog box, in which you will specify a name for the new .inf file for the converted images.

Begin

Carries out the conversion of the PI MultiViewer images to separate, MetaFluor-compatible images.

Cancel

Cancels the Convert PI MultiViewer Experiment command.

Define Split in Image - Dialog Box Options

Select an Image from the Experiment

Selects a suitable source image for viewing while adjusting the location of the split line between the MultiViewer image pair.

Use Automatic Scaling

Scales the 16-bit source image automatically to an 8-bit display, setting the lowest intensity value in the original image to gray level 0 and setting the highest intensity value to gray level 255. When this check box is selected, *Low* and *High* will be unavailable. Clearing this check box will enable the use of *Low* and *High.*

Low

Selects a grayscale intensity value in the source image that will be scaled to gray level 0 in an 8-bit display. This option will be unavailable if *Use Automatic Scaling* has been selected.

High

Selects a grayscale intensity value in the source image that will be scaled to gray level 255 in an 8-bit display. This option will be unavailable if *Use Automatic Scaling* has been selected.

OK

Accepts the split line location setting, closes the Define Split in Image dialog box, and opens the Align Top and Bottom dialog box.

Cancel

Cancels the Convert PI MultiViewer Experiment command.

Align Top and Bottom/Align Left and Right - Dialog Box Options

View Alignment Image As

Selects the method for viewing the alignment between the superimposed MultiViewer image pair:

Subtraction subtracts the grayscale values of the second image from those the first, pixel by pixel.

Average takes an average of the grayscale values of corresponding pixels in the two images.

Color renders the first half-image in red and the second is rendered in green.

Ratio is constructed by "ratioing" the pixel values in first image by those of the second image.

Align Images (Left, Right, Top, Bottom)

These four buttons align the images by "nudging" the second image (bottom, or right) relative to the first (top, or left), one pixel at a time.

Current Offset

Indicates the current X and Y axis displacement, in pixels, of the second image with respect to the first image.

Select an Image from the Experiment

This slider and text box allows you to select an image frame from the original experiment for display while aligning the two paired images.

Use Automatic Scaling

Scales the 16-bit source image automatically to an 8-bit display, setting the lowest intensity value in the original image to gray level 0 and setting the highest intensity value to gray level 255. When this check box is selected, *Low* and *High* will be unavailable. Selecting this check box will enable the use of the *Low* and *High* options.

Low

Selects a grayscale intensity value in the source image that will be scaled to gray level 0 in an 8-bit display. This option will be unavailable if *Use Automatic Scaling* has been selected.

High

Selects a grayscale intensity value in the source image that will be scaled to gray level 255 in an 8-bit display. This option will be unavailable if *Use Automatic Scaling* has been selected.

OK

Accepts the alignment settings, closes the Align Top and Bottom (or Align Left and Right) dialog box, and opens the Convert MultiViewer Experiment dialog box.

Cancel

Cancels the Convert PI MultiViewer Experiment command.

Twain Configure (Utilities Menu)

Selects a TWAIN-compliant device for image acquisition and specifies whether to use the device's user interface.

Drop-in: TWAINCFG

Use this command to select a different TWAIN device and user interface option for image acquisition without the need for exiting MetaFluor to use the external Video Driver Manager program. The Configure Twain Driver dialog box that this command displays is the same as that used by the Video Driver Manager program.

The dialog box displays provides a list of the TWAIN-compliant devices that you have installed on your system. This list will reflect the TWAIN device files that reside on your system, regardless of whether the equipment is still actually connected. Examples of TWAIN-compliant devices include the certain video cameras, CCDs, and flat-bed scanners.

A typical use of this command is to select the *Show Device User Interface* check box so that the acquisition options for the TWAIN device will appear before each acquisition. Most TWAIN devices can save the acquisition settings from their respective device user interfaces. You can use this feature to fine-tune your acquisition settings, save the settings, and then clear the *Show Device User Interface* check box in this dialog box and proceed to acquire your images in non-interactive mode.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Introduction to the MetaMorph Video Driver Manager

Configuring Use of a TWAIN-Compliant Device

To configure use of a TWAIN device for image acquisition, use the following procedure:

Step	Action
1	From the Utilities menu, choose Twain Configure. The Configure Twain Driver dialog box will appear.
2	From the *Installed Devices* table, select the TWAIN device you want to use.
3	Select the *Show Device User Interface* check box so that a check mark appears in it. Then choose *OK.* The Configure Twain Driver dialog box will close.
4	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel will appear.
5	Choose *Acquire.* The user interface dialog box for your selected TWAIN-compliant device will appear.
6	Make any adjustments to the acquisition settings that are necessary, and acquire and transfer some images through the use of the device's user interface.
7	If you want to acquire images using the device's user interface, you are done. Simply proceed to acquire your experimental images using the user interface. **OR** If you want to acquire images automatically in non-interactive mode, choose Twain Configure again from the Utilities menu. The Configure Twain Driver dialog box will reappear.
8	Clear the *Show Device User Interface* check box. Then choose *OK.*
9	When you are ready to acquire your images automatically, choose *Acquire* from the Experiment Control Panel. Remember to select the *Save Images, Save Ratios,* and *Log Data* check boxes, as necessary.

Twain Configure - Dialog Box Options

Installed Devices

Lists the TWAIN-compliant devices (EXAMPLES: FlashPoint video acquisition board, Spot cameras, scanners, etc.) that you have installed on your system. Select the device that you want to use from this list before acquiring images with the TWAIN device.

Show Device User Interface

Selecting this check box will configure your system to display the TWAIN device's interface when you choose the acquisition command. You should acquire an image in this fashion at least once to adjust your image acquisition settings before you subsequently acquire images in non-interactive mode with this check box cleared.

OK

Accepts your selection of a TWAIN-compliant device from the *Installed Devices* table and closes the Configure Twain Driver dialog box.

Cancel

Cancels any changes you have made to the settings in the Configure Twain Driver dialog box and closes the dialog box.

Calibration Menu

Acquire Calibration Standards (Calibration Menu)

Acquires or loads calibration standards.

Use the Acquire Calibration Standards command when you want to acquire or load calibration standards. A calibration standard is an image or set of images that represent a known value, such as a pH or ion concentration.

This command maintains a table for you to keep track of your calibration standards.

See Also:

Equation Calibration in situ

Titration Calibration in situ

Quench Calibration

Acquiring Calibration Standards

To acquire the calibration standards, use the following procedure:

Step	Action
1	From the Calibration menu, choose Acquire Calibration Standards. The Acquire Calibration Standards dialog box will appear.
2	From the *What Is Being Calibrated?* drop-down list, select the image you want to calibrate. If you are using the Equation or Titration Calibration methods, you can select either a wavelength image or a ratio image. If you are using the Quench Calibration, you will only be able to select a wavelength image.
3	If needed, you can change the name of the calibrated image series by typing a new name in the *Name* text box.
4	From the *Calibration Mode* group, select the desired calibration mode: *Equation, Titration,* or *Quench.*
5	If you have previously saved a set of calibration standards and wish to use it now, choose *Load Standards* and select the icon for the desired .cal file from the Load Calibration Standards dialog box that appears. Then choose *Open* to return to the Acquire Calibration Standards dialog box. Now skip to Step 13. **OR** If you still need to configure a calibration, continue with Step 6.
6	The scroll bar, buttons, *Value* options, and *Comments* text boxes form a table that keeps track of the calibration reference images stored in memory. This table can contain up to 16 entries. Choose the button numbered with the calibration reference image you intend to acquire or load. The button will change from a number to ">>" and a corresponding "<<" will appear at the right side of the table to indicate that this row in the table is the active row.
7	Choose *Acquire* to acquire an image or image pair for the active table row if you want to acquire the reference image or pair from video. If you are about to overwrite an existing calibration table entry, a warning message will appear, asking you to verify the overwrite. **OR** Choose *Load* if you want to load a reference image from the hard drive. Select the desired

image you want to load.

Note: If *Ask Whether Backgrounds Should Be Subtracted* is selected in the Calibration Preferences dialog box, you will be asked to indicate whether the background reference image should be subtracted from the data image (or image pair) that you are loading, and you will be allowed to load in a new background.

OR

Choose *Current* to use the current image (the image that was last acquired or loaded before you opened the Acquire Calibration Standards dialog box) for a calibration reference.

Note: If you need to delete the selected table row, choose *Delete.*

8   After you have loaded or acquired the image, type the appropriate value for the image in the corresponding *Value* text box.

If you are using Titration Calibration, you need to use the concentration that this image represents, such as pH value or ion concentration, in the *Value* text box. If you are using the Equation Calibration, you need to indicate, using the *Lo* or *Hi* radio button, whether this image corresponds to a low (minimum) concentration or a high (maximum) target ion concentration. The Quench Calibration does not require a value.

9   You can type a description of the image in the *Comments* text box if needed.

10   If you want to display the calibrated images associated with the selected table row, select the *View Calibration Images* check box.

11   Repeat Steps 6 - 11 for each calibration reference image you want to load. The *Status* line will indicate how many images are loaded and how many table rows are in use. You can use up to 16 rows (16 calibration data points) in the table.

12   If you want to save the calibration, choose *Save Standards* and type a name for the calibration (.cal) file in the *File Name* text box of the Save Calibration Standards dialog box that appears. Then choose *Save* to return to the Acquire Calibration Standards dialog box.

13   Choose *OK.*

Acquire Calibration Standards - Dialog Box Options

Load Standards

Loads a set of calibration standards that was saved to disk using *Save Calibration Standards.* This command opens the Load Calibration Standards dialog box.

Save Standards

Allows you to save the current set of calibration standards to disk. This command opens the Save Calibration Standards dialog box.

What Is Being Calibrated?

Specifies the image that you want to calibrate. You can select either a wavelength image or a ratio image.

Name

Provides an alternative name for the calibrated image series.

View Calibration Images

Displays the calibration image(s) associated with the selected table row. When this box is checked, the images will appear in image windows on your computer monitor.

Calibration Mode

Specifies the calibration mode that you want to use. You can select *Equation, Titration, or Quench.*

Correct All Images

Opens the Correct All Images dialog box, which corrects the calibration images for background and shading.

Slider

Displays additional rows of buttons, *Values,* and *Comments* in the table of calibration standards.

Buttons (1 through 16)

Indicates the row number in the table of calibration standards. The table can consist of up to 16 entries. The number on the button will change to >> if it is the active row in the table.

Value

If you are applying the Titration Calibration, the *Value* is the concentration that the current image represents, such as a pH value or ion concentration. If you are applying the Equation Calibration, select *Lo* to indicate that the image corresponds to a low (minimum) concentration or select *Hi* to indicate that it corresponds to a high (maximum) concentration. The Quench Calibration does not require a value.

Comments

Allows you to provides a description of the image.

>> and **<<**

Indicates the active row in the table.

Acquire

Acquires an image or image pair for the active table row from video. All images acquired using *Acquire* will use the existing acquisition settings (such as illumination settings, background subtraction, and shading correction).

Load

Loads a reference image from your hard disk for the active table row.

Current

Uses the current image (the image that was last acquired or loaded before you opened the Acquire Calibration Standards dialog box) for the active table row.

Delete

Deletes the selected table row.

OK

Closes the dialog box.

Equation Calibration in situ (Calibration Menu)

Configures the Kd and Viscosity constants for Equation Calibration in situ. Displays the calibration curve.

Use the Equation Calibration in situ command to set the Kd and Viscosity constants when you want to calibrate by equation. The Equation Calibration in situ command requires input of these constants, which it uses to calculate the calibration equation. After you have entered the constants you want to use, you can display the calibration curve.

The calibration equation is as follows:

$$Concentration = K_d \left(\frac{S_{f2}}{S_{b2}} \right) \left(\frac{R - Visc \cdot R_{Min}}{Visc \cdot R_{Max} - R} \right)$$

Where:

 Kd is the dissociation constant for the fura-2/calcium complex,

 Viscosity is a correction factor that when used, is typically between 0.7 and 0.85. When not used, choose a value of 1,

 Conc is the resulting concentration of the ion being measured (for example, Calcium) as a result of applying the calibration equation,

 Sf2 is the fluorescence intensity at the denominator wavelength in an ion-free environment. For fura-2, this will be the intensity of the 380nm image in a calicum-free calibration image,

 Sb2 is the fluorescence intensity at the denominator wavelength in an ion-bound environment. For fura-2, this will be the intensity of the 380nm image in a calicum-saturated calibration image,

 R is the experimentally measured ratio of intensities during 340-nm and 380-nm excitation,

 Visc is the intracellular viscosity correction factor,

 RMin is the ratio (340/380) of the intensities of the zero-calcium calibration reference images, and

 RMax is the ratio (340/380) of the intensities of the maximal calcium reference images.

Note: You must select *Equation* as the calibration mode from the *Calibration Mode* group in the Acquire Calibration Standards dialog box prior to using this command.

 See Also:

Equation Calibration in vitro

Configuring Equation Calibration in situ

To configure the Equation Calibration in situ, use the following procedure.

Note: You must select *Equation* as the calibration mode from the *Calibration Mode* group in the Acquire Calibration Standards dialog box prior to using this command.

Step	Action
1	From the Calibration menu, choose Equation Calibration in situ. The Equation Calibration in situ dialog box will appear.
2	In the *Kd Value* text box, type the appropriate dissociation constant for your experiment . For a fura-2 experiment performed at 37 degrees in 1 mM Mg++, Kd has been calculated to be approximately 224 nM.
3	If necessary, you can type a viscosity correction value in the *Viscosity* text box. Viscosity correction is a fractional value up to and including 1.0 (the default value). **Note:** This option will not appear unless you selected *Ratio* from the *Calibrate* group in the Acquire Calibration Standards dialog box.
4	After you have entered the parameters, you can choose *Show Calibration Curve* to view the calibration curve and verify that is appropriate. The dialog box will expand to show the graph of the calibration curve and an additional option, *Select the Region to Plot.* If everything has been configured correctly, the *Status* line will read "Equation Calibration Is Configured OK."
5	The curve will be shown for the first region of interest. If you want to see the curve for a different region of interest, use the *Select the Region to Plot* spin box to select the number for a different region.
6	The Y-axis of the calibration curve will range from the minimum ratio to the maximum ratio, as determined by the *Min. Ratio* and *Max. Ratio* values in the Image Display Controls dialog box. The minimum ratio in this dialog box may be below the actual RMin value, or the maximum ratio may be above the actual RMax value. If so, values outside of RMin and RMax will generate an error message that is shown in the Status window, and those values will not be plotted on the graph. You should close the Equation Calibration in situ dialog box and open the Image Display Controls dialog box to adjust the minimum and maximum ratio, and then plot the calibration again.

7   Choose *Close* when you have finished.

Equation Calibration in situ - Dialog Box Options

Kd Value

Enter the appropriate ion-indicator dissociation constant (Kd) value for your experiment. For a fura-2/calcium experiment performed at 37 degrees C in 1 mM Mg++, Kd has been calculated to be 224 nm (Grynkiewicz, et al., *J. Biol. Chem.* 260: 3440, 1985).

Viscosity

Viscosity correction is a fractional value up to and including 1.0. More information about viscosity correction is available in the article by M. Poenie (*Cell Calcium* 11: 85, 1990).

Calibration Equation

Displays the calibration equation that is being used.

Status

Displays the status of the calibration equation and indicates whether there are additional steps that you need to perform before the Equation Calibration in situ can be completed.

Show Calibration Curve

Displays the calibration curve. When the calibration curve is being displayed, the Y-axis will range from the minimum ratio to the maximum ratio, as determined by the Image Display Controls dialog box.

Definition of Terms

Indicates the names and definitions of the terms used in the Calibration Equation. Double click the name of the term to display a detailed definition.

Close

Closes the dialog box.

Equation Calibration in vitro (Calibration Menu)

Configures the Equation Calibration in vitro. Displays the calibration curve.

Use the Equation Calibration in vitro command when you are measuring a homogenous sample and you do not need separate equations for each region of interest. When using this command, you will only need to enter the known equation constants (typically calculated from a previous session or experiment), and MetaFluor will create a single calibration equation, which it uses for any region being measured.

The Equation Calibration in vitro command is useful if you are measuring solutions for in vitro calibration, instead of living specimens. This method will save time and memory because the calibration reference images do not need to be acquired or maintained in memory.

This method of calibration is also recommended when you are not using a perfusion system and must add drugs by pipette. Because the other Equation Calibration method performs a pixel-by-pixel calculation, errors may crop up due to the high likelihood of cell movement during infusions with a pipette. The Equation Calibration in vitro minimizes this source of error.

See Also:

Equation Calibration in situ

Configuring Equation Calibration in vitro

To configure the Equation Calibration in vitro, use the following procedure:

Step	Action
1	From the Calibration menu, choose Equation Calibration in vitro. The Equation Calibration in vitro dialog box will appear.
2	Type the Kd and viscosity values appropriate for your experiment in the *Kd Value* and *Viscosity* text boxes.
3	Type the Equation Calibration values for Sf2, Sb2, RMin, and RMax in the appropriate *Equation Calibration Variable* text boxes. These can be values that you have obtained during previous experiments. **OR** Choose *Set from Image.* A message window will appear, informing you that Sf2, Sb2, RMin, and RMax will be set from the low and high concentration reference images loaded in the Acquire Calibration Standards procedure. Choose *OK.*
4	After you have selected the equation constants and variables, you can choose *Show Calibration Curve* to view the calibration curve graph. The button text will change to *"Hide Calibration Curve,"* and the dialog box will expand to display the graph of the calibration curve. If everything has been configured correctly, the *Status* line will read "Equation Calibration Is Configured OK." **Note:** The *Min. Ratio* and *Max. Ratio* options in the Image Display Controls dialog box must be set above and below the RMin and RMax values, respectively; otherwise you will not be able to view the entire calibration curve.
5	Choose *Close* when you have finished.

Equation Calibration in vitro - Dialog Box Options

Kd Value

Type the appropriate Kd value for your experiment.

Viscosity

Viscosity correction is a fractional value up to and including 1.0. More information about viscosity correction is available in the article by M. Poenie *(Cell Calcium* 11: 85, 1990).

Calibration Equation

Displays the calibration equation used by this command.

Equation Calibration Variables

Type the variables for Sf2, Sb2, RMin, and RMax in the appropriate text boxes. These values can be obtained from previous experiments.

Set from Image

Use this option to calculate the equation variables from Low and High concentration images that were already measured with the Acquire Calibration Standards command rather than typing the values in the *Equation Calibration Variables* text boxes.

Show Message

The *Show Message* check box only applies to the *Set from Image* button. If you choose the *Set from Image* button and you have selected the *Show Message* check box, a dialog box will appear which tells you what MetaFluor is about to do. If you don't have the *Show Message* check box selected, MetaFluor will complete the measurement without alerting you that it is doing so. The *Set from Image* button is enabled only if you have images in the *Acquire Calibration Images* table. You need to have the table set to *Equation Calibration* and have at least one *Lo* and one *Hi* image pair acquired. When you choose *Set from Table,* MetaFluor will calculate the *Sf2, Sb2, RMin,* and *RMax* variables for you by measuring the images in the *Acquire Calibration Images* table.

Status

Displays the status of the calibration and indicates any additional steps that you need to do before the calibration is completed.

Show Calibration Curve

Displays the calibration curve. You will not be able to view the entire calibration curve unless the *Min. Ratio* and *Max. Ratio* options in the Image Display Control dialog box are above and below the RMin and RMax values, respectively.

Hide Calibration Curve

Hides the calibration curve.

Definition of Terms

Indicates the names and definitions of the terms used in the Calibration Equation. Double click the name of the term to display a detailed definition.

Close

Closes the dialog box.

Titration Calibration in situ (Calibration Menu)

Configures the Titration Calibration in situ. Displays the calibration curve.

Use the Titration Calibration in situ command to select the calibration method and curve-fitting parameters when you want to calibrate by titration. The Titration Calibration in situ command consists of a curve-fit between known concentrations and their corresponding ratios or intensities. You first must collect data for this curve-fit by using the Acquire Calibration Standards command.

Note: You must select *Titration* as the calibration mode from the *Calibration Mode* group in the Acquire Calibration Standards dialog box prior to using this command.

Configuring Titration Calibration in situ

To configure the Titration Calibration in situ, use the following procedure.

Note: You must select *Titration* as the calibration mode from the *Calibration Mode* group in the Acquire Calibration Standards dialog box prior to using this command.

Step	Action
1	From the Calibration menu, choose Titration Calibration in situ. The Titration Calibration in situ dialog box will appear.
2	Select the desired curve-fit from the *Curve Fit* drop-down list. If your standards range over just a few levels (e.g., pH 6.0 - 8.0, or 30 - 300 nM), *Linear Interpolation* may be more than adequate.
3	If you want your calibration curve to extend beyond the first and last data points, select *Yes* from the *Extrapolate* group. Otherwise, select *No.*
4	If necessary, you can enforce a minimum and/or maximum limit to the curve by selecting the *Enforce Maximum Limit* and/or *Enforce Minimum Limit* check boxes. You should then type a value in the *Value* text box. This is particularly recommended if you enabled extrapolation in the preceding step.
5	Choose *Show Calibration Curve* to view the calibration curve. The button text will change to *"Hide Calibration Curve,"* and the dialog box will expand to display the graph of the calibration curve and an additional option, *Select Region to Plot.* Ratio values will fall on the Y-axis, ranging from the minimum ratio to the maximum ratio as defined by the *Min. Ratio* and *Max. Ratio* values in the Image Display Controls dialog box. Calibrated values will fall on the X-axis. All of the known points will be drawn as circles on the graph. The calibration curve will appear as a red trace line.
6	If you want to view the curve for a different region of interest, use the *Select the Region to Plot* spin box to select the number of the desired region of interest.
7	Choose *Close* when you have finished.

Titration Calibration in situ - Dialog Box Options

Curve Fit

Specifies the curve-fit to be used for the Titration Calibration in situ:

Linear Interpolation draws a curve that connects each point to the previous and next point.

Polynomial Interpolation computes a polynomial that best fits the data.

Line of Best Fit finds a straight line that falls closest to all of the data points.

Line of Best Log finds a log line that falls closest to all of the data points.

3rd Degree Polynomial computes a third-degree polynomial that best fits the data.

4th Degree Polynomial computes a fourth-degree polynomial that best fits the data.

Extrapolate

Select *Yes* to extrapolate beyond the first and last data point. The curve will be extended out to the left of the first data point and to the right of the last data point. The curve is extended simply by propagating the slope of the line between the last and penultimate points.

If *No* is selected from the *Extrapolate* group, the curve will only cover the range between the first and last data points, and subsequently measured points that fall outside of this range will not be calibrated.

Enforce Maximum Limit and **Enforce Minimum Limit**

Use these two options to set limits for the curve. When these options are selected, the curve will not go beyond the values selected in the *Value* text boxes.

EXAMPLE**:**
To calibrate pH, you can select a *Minimum Limit* of *5* and a *Maximum Limit* of *9.* This would ensure that the calibrated data points remain within the pH range of 5.0 - 9.0.

Status

Displays the status of the titration equation and indicates whether there are additional steps that you need to perform before the Titration Calibration in situ can be completed.

Show Calibration Curve

Displays the calibration curve. Ratio values will fall on the Y-Axis, ranging from the minimum ratio to the maximum ratio as defined in the Image Display Controls dialog box. Calibrated values will fall on the X-axis. All of the known points will be drawn as circles on the graph. The calibration curve will appear as a red trace line. You can choose which region of interest's calibration is displayed by changing the region selector at the top of the graph.

Close

Closes the dialog box.

Titration Calibration in vitro (Calibration Menu)

Configures the Titration Calibration in vitro. Displays the calibration curve.

This command allows you to enter the measured values and calibrated (actual) values for a titration calibration manually when you have obtained these values from another source. If you have already set up calibration reference images using the Acquire Calibration Standards dialog box, MetaFluor can measure these images and determine the measured values from them. You can then enter the calibrated values that correspond to these measured values in the Titration Calibration in vitro table. Once you have configured the Titration Calibration in vitro you can display the calibration curve.

This method of calibration is also recommended when you are not using a perfusion system and must add drugs by pipette. Because the other Titration Calibration method performs a pixel-by-pixel calculation, errors may crop up due to the high likelihood of cell movement during infusions with a pipette. The Titration Calibration in vitro minimizes this source of error.

See Also:

Titration Calibration in situ

Configuring Titration Calibration in vitro

Manually Configuring Titration Calibration in vitro

Configuring Titration Calibration in vitro Using Reference Images

Manually Configuring Titration Calibration in vitro

To configure the Titration Calibration in vitro manually, use the following procedure:

Step	Action
1	From the Calibration menu, choose Titration Calibration in vitro. The Titration Calibration in vitro dialog box will appear.
2	Select *Manually Configure* from the *Data Source* group to enter values manually in the table that you have collected from another source.
3	In the *Values* are box, choose the ratio or wavelength to which you want to apply this calibration.
4	Select the number of entries for the table using *Entries.* **Note:** You must have at least two entries.
5	Using the data that you have collected from other sources, enter the measured value and the calibrated value for each entry in the *Intensity* (or *Ratio)* and *Cal. Value* text boxes.
6	Specify the type of values for the table using the *Entered Value Is* group. You can select an intensity wavelength or a ratio.
7	Choose *Curve Fit.* The Titration Calibration in vitro Graph dialog box will appear.
8	You may need to reconfigure the graph. To do so, select the desired curve-fitting method from the *Curve Fit* drop-down list. If you want to extrapolate the points beyond the first and last values, select *Yes* in the *Extrapolate* group. If you know that the calibrated values should not extend beyond a minimum and/or maximum point, you can use *Enforce Minimum Limit* and *Enforce Maximum Limit* to set them.
9	If you are satisfied with the calibration, choose *OK* to set it and close both dialog boxes. **OR** Choose *Cancel* to return to the Titration Calibration in vitro dialog box. Repeat Steps 2 - 8.

Configuring Titration Calibration in vitro Using Reference Images

To configure the Titration Calibration in vitro using reference images, use the following procedure.

Note: You must define regions using the Define Regions for Measurement command (Graphs menu) and acquire or load the reference images using the Acquire Calibration Standards command prior to using this command.

Step	Action
1	From the Calibration menu, choose Titration Calibration in vitro. The Titration Calibration in vitro dialog box will appear.
2	Select *Use Data in Acquire Calibration Standards* from the *Data Source* group. The calibrated values from the *Value* text boxes in the Acquire Calibration Images dialog box will appear in the table.
3	If you know the measured value, type it in the *Intensity* (or *Ratio)* text box. **OR** If you do not know the measured value, choose the button in the *Measure* column of the table *(1, 2, 3,* and so on) to measure the region of interest. MetaFluor will determine the value from the region and display it for you.
4	Repeat Step 3 for each row in the table.
5	Choose *Curve Fit.* The Titration Calibration in vitro Graph dialog box will appear.
6	You may need to configure the graph. To do so, select the desired curve-fitting method from the *Curve Fit* drop-down list. If you want to extrapolate the points beyond the first and last values, select *Yes* in the *Extrapolate* group. If you know that the calibrated values should not extend beyond a minimum and/or maximum point, you can select *Enforce Minimum Limit* and *Enforce Maximum Limit* to set fixed end-points.
7	If you are satisfied with the calibration, choose *OK* to set it and close both dialog boxes. **OR** Choose *Cancel* to return to the Titration Calibration in vitro dialog box. Repeat Steps 2 through 6.

Titration Calibration in Vitro - Dialog Box Options

Data Source

Select *Use Data in Acquire Calibration Standards* if you want to measure calibration reference images that have already been acquired or loaded using the Acquire Calibration Standards command. This automatically loads the entries for the *Cal. Value* column of the table.

Select *Manually Configure* if you want to enter values manually in the table that you have collected from another source.

Values Are

This option appears only if you have selected *Manually Configure* as the *Data Source*. It enables you to associate the calibrations for in vitro titration with a specific ratio or wavelength. Select the ratio or wavelength you want from the *Values are* drop-down list.

Entries

This option appears only if you have selected *Manually Configure* as the *Data Source.* This allows you to set the number of entries in the table. You must have at least two entries. The number of entries depends on the number of calibration standards you have set up.

Status

Displays the status of the calibration and indicates any additional steps that you need to take before the calibration is completed.

Measure

This option is available only if you have selected *Use Data in Acquire Calibration Standards* as the *Data Source.* Use the button in this column to measure the region in the calibration reference image associated with that particular row of the table and determine the *Intensity/Ratio* value for you.

Intensity/Ratio

Enter the measured (intensity or ratio, as applicable) value in this text box. If you have selected *Use Data in Acquire Calibration Standards,* you can choose *Measure* if you want MetaFluor to measure the region of interest in the associated calibration reference image and then determine this value for you.

Cal. Value

This is the calibrated (actual) value. If you are configuring the Titration Calibration in vitro manually, you will need to obtain this from some other source. If you have selected *Use Data in Acquire Calibration Standards,* these values will be obtained from the Acquire Calibration Standards by MetaFluor.

Entered Value Is

This option will be available only if you have selected *Manually Configure* as the *Data Source*. Specify either an intensity wavelength or a ratio.

Curve Fit

Displays the calibration curve. You can select the desired curve-fitting method from the *Curve Fit* drop-down list.

If you want to extrapolate the points beyond the first and last values, select *Yes* in the *Extrapolate* group. The curve will be extended out to the left of the first data point and to the right of the last data point. The curve is extended simply by propagating the slope of the line between the last and penultimate points.

If you select *No* from the *Extrapolate* group, the curve will only cover the range between the first and last data points, and subsequently measured points that fall outside of this range will not be calibrated.

If you know that the calibrated values should not extend beyond a minimum and/or maximum point, you can use *Enforce Minimum Limit* and *Enforce Maximum Limit* to set them.

Choosing *OK* will complete the Titration Calibration in vitro and close the Titration Calibration in vitro dialog box. *Cancel* allows you to return to the Titration Calibration in vitro dialog box.

Close

This closes the dialog box without completing the Titration Calibration in vitro. You must display the *Curve Fit* to complete the Titration Calibration in vitro.

Quench Calibration (Calibration Menu)

Configures the Quench Calibration. Displays the calibration curve.

This method of calibration is used exclusively for single-wavelength dyes, such as Calcium Green and fluo-3. Use the Quench Calibration command to select the appropriate constants when you want to calibrate by quench equation. This command requires Multiplier and Divisor constants in addition to the standard Kd constant (for derivation, see Kao, et al., *J. Biol. Chem.,* 264: 8179, 1989). These are used when calibrating the calibration equation.

Quench calibration is based on the principle that, when a saturating concentration of a heavy metal (e.g., 2 mM Mn++) is added to the preparation and the cells are made permeable, dye fluorescence will be quenched to an intensity that is roughly 20% of maximum. Metal-free dye in lysed cells is known to have a fluorescence that is 1/40th of maximum. From these assumptions, the FMax can be derived from the following formula

$$F_{Max} = \frac{\left(F_{Mn} - F_{bkg}\right)}{0.2} + F_{bkg}$$

Subsequently, FMin can be calculated from the formula

$$F_{Min} = \frac{\left(F_{Max} - F_{bkg}\right)}{40} + F_{bkg}$$

Finally, the ion concentration is automatically calculated by MetaFluor as

$$Conc = \frac{K_d\left(F - F_{Min}\right)}{\left(F_{Max} - F\right)}$$

Note: This command can only be used for single-wavelength dye calibrations. It does not apply to ratiometric calibrations.

Note: You must select *Quench* as the calibration mode from the *Calibration* group in the Acquire Calibration Standards dialog box prior to using this command.

Configuring the Quench Calibration

To configure the Quench Calibration, use the following procedure.

Note: You must select *Quench* as the calibration mode from the *Calibration Mode* group in the Acquire Calibration Standards dialog box prior to using this command.

Step	Action
1	From the Calibration menu, choose Quench Calibration. The Quench Calibration dialog box will appear.
2	Type the appropriate ion-indicator dissociation constant (Kd) for your experiment in the *Kd Value* text box.
3	The Quench Calibration uses the equations listed in the right half of the dialog box to approximate low and high concentration images. After these are obtained, it will apply the Equation Calibration algorithm.
	The Quench Calibration equations require that the *Multiplier M* and *Divisor D* variables be specified. Suggested values are *5* and *40,* respectively.
4	Choose *Show Calibration Curve* to view the calibration curve. The button text will change to *"Hide Calibration Curve,"* and the dialog box will expand to display the graph of the calibration curve and an additional option, *Select the Region to Plot.*
	If everything has been configured correctly, the *Status* line will read "Quench Calibration Is Configured OK."
5	If you want to view the curve for a different region of interest, use the *Select the Region to Plot* spin box to select the number for the region you want to specify instead.
6	Choose *Close* when you have finished.

Quench Calibration - Dialog Box Options

Kd Value

Type the appropriate Kd value for your experiment.

Multiplier M

Specifies the multiplier variable, M, used by the calibration equation. This value must be specified. The value *5* is recommended.

Divisor D

Specifies the denominator variable, D, used by the calibration equation. This value must be specified. The value *40* is recommended.

Status

Displays the status of the titration equation and indicates whether there are additional steps that you need to perform before the Quench Calibration can be completed.

Calibration Equation

Displays the calibration equation used by the Quench Calibration command.

Show Calibration Curve

Displays the calibration curve.

Close

Closes the dialog box.

Calibration Map (Calibration Menu)

Applies a calibration to an experimental image, generating a custom look-up table that assigns a specific color to a particular ion concentration or pH value. The "calibration map" that is displayed will correspond to the currently active experimental image.

Use this command to generate a calibration map which displays the image in calibrated values, where the difference in color between pixels directly relates to the difference in concentration. This command allows you to visualize the relationships of ion concentration in the specimen.

If you want to apply a calibration to an entire series of experimental images, you should first apply this command, and then perform the procedure for the Save Calibration Maps command.

QUICK TIP: To hide the Image Window Toolbar, right-click on the image and choose Hide Image Window Toolbar from the pop-up shortcut menu that appears.

See Also:

Save Calibration Maps

Generating a Calibration Map

To generate a calibration map, use the following procedure:

Step	Action
1	Select the image you want to convert into a calibration map so that it is the active image.
2	From the Calibration menu, choose Calibration Map. The Calibration Map dialog box will appear.
3	Type the expected minimum and maximum calibrated values (ion concentration or pH) in the *Minimum Calibrated Value* and *Maximum Calibrated Value* text boxes.
4	Select the desired look-up table from the *Look-up Table* drop-down list. *Rainbow* is the recommended option.
5	You can draw a scale bar on the calibration map as a key. To do so, select *Draw Scale Bar.* Then choose *Configure Scale Bar.* The Draw Scale Bar dialog box will appear.
	Type the desired title in the *Title* text box. This should be a short name, such as the default "Conc." Select the desired drawing colors using *Background Color* and *Foreground Color.*
	The Calibration Scale Bar is configured through your choice of labels, rather than automatically. Type the first label in the *Label* text box and choose *Add* so that it appears in the list box. Repeat for the remainder of the desired value labels. You can use *Change, Delete, Sort Up,* and *Sort Down* to change the list.
	Use *Location* to select the desired location on the image for the scale bar. Choose *Close* when you have finished.
6	Choose *Generate Image* to create the calibration map. When MetaFluor has finished computing the map, the calibration map will appear on the screen.
7	If you wish, you can save the Calibration Map by choosing *Save Image.*
8	Choose *Close* when you have finished.

Calibration Map - Dialog Box Options

Minimum Calibrated Value

Type the minimum calibrated value that you expect to be in the image which you want to convert to a calibration map.

Maximum Calibrated Value

Type the maximum calibrated value that you expect to be in the image which you want to convert to a calibration map.

Look-up Table

Specifies the look-up table to use for the calibration map. *Rainbow* is the recommended option.

LUT File

This option will be available when *Custom* is selected from the *Look-up Table* list. Use this option to select a custom look-up table from the Select LUT dialog box. Select the desired file name from the *File Name* list.

Draw Scale Bar

Draws a scale bar on the calibration map as a key.

Configure Scale Bar

Configures the scale bar. This command opens the Draw Scale Bar dialog box which allows you to configure the title, scale bar colors, labels, and location of the scale bar.

Generate Image

Displays the calibration map.

Save Image

Saves the calibration map.

Close

Closes the dialog box.

Save Calibration Maps (Calibration Menu)

Converts a series of calibration images to calibration maps and saves them.

Use this command when you want to save calibration images as calibration maps using sequential file names. You can create movies from the calibration maps with the Play Movie from Disk drop-in command (Utilities menu) or by using MetaMorph.

Prior to applying this command, you must use the Calibration Map command to calibrate the first experimental image. Following that, applying the Save Calibration Maps command will generate and save calibration maps for the entire experiment.

See Also:

Calibration Map

Saving Calibration Maps

To generate and save multiple calibration maps from an entire experiment, use the following procedure:

Step	Action
1	From the Calibration menu, choose Save Calibration Maps. The Save Calibration Maps dialog box will appear.
2	Type a file name in the *Base File Name* text box. For example, if the base file name is "Calib," images will be saved as Calib0001.tif, Calib0002.tif, and so on.
3	Use *Sequence* to select the starting number for the sequence.
4	Choose *Directory* to specify the folder for saving the images. The Browse for Folder dialog box will appear. Select the desired folder and then choose *OK* to return to the Save Calibration Maps dialog box.
5	Select the *Generate and Save Calibration Map After Each Cycle* check box if you want to generate a calibration map and save it using a sequential file name after each cycle. The sequence number will be incremented after each cycle.
6	Choose *OK.*
7	After you have set up the Save Calibration Maps sequence, you can open the Experiment Control Panel and "play back" the experiment. The calibration maps will be saved after each ratio cycle is completed.

Save Calibration Maps - Dialog Box Options

Base File Name

The base file name for the calibration maps that will be saved.

Sequence

Specifies the starting number of the sequence.

Directory

Opens the Browse for Folder dialog box, from which you can select the folder in which to save the images.

Generate and Save Calibration Map After Each Cycle

When this option is selected, MetaFluor will generate a calibration map and save it after each cycle, using a sequential file name. The sequence number will be incremented after each cycle.

OK

Closes the dialog box.

Save Calibration Table (Calibration Menu)

Saves the current ratio or calibration scale as a .gry file.

Drop-in: SAVECAL

Use this command when you want to save a ratio image or calibration scale to disk. The calibration table can then be used in MetaMorph and applied to a grayscale TIFF image with the Calibrate Gray Levels command (Measure menu) for measurement of brightness, optical density, etc.

Note: If the system has not been calibrated, the ratio scale will be saved. Otherwise the current calibration scale is saved.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Saving Calibration Tables

To save a calibration table, use the following procedure:

Step	Action
1	From the Calibration menu, choose Save Calibration Table. The Save Calibration Table dialog box will appear.
2	From the *Ratio Image* list, select the ratio image to use for the calibration table.
3	Choose *Select File.* The Save Calibration Table dialog box will appear.
4	Type a new file name in the *File Name* text box for the first of the two images, or select an icon for an existing file.
	If the desired folder is not listed at the top of the dialog box, use the *Save In* drop-down list box or Up One Level icon button to change to the correct location. Then select a file name.
5	Choose *Save.* The Save Calibration Table dialog box will close.
6	From the Save Calibration Table dialog box, choose *Save.* The ratio scale or calibration scale will be saved as a calibration table in a file with a *.gry extension, and the dialog box will close.

Save Calibration Table - Dialog Box Options

Ratio Image

Selects the ratio image *(Ratio 1* or *Ratio 2)* from which to obtain the calibration or ratio scale (depending on whether or not the image has been calibrated).

Select File

Selects a file name for saving the calibration table. You can save the table under a new name or you can overwrite an existing file.

Save

Calculates ion concentrations for all possible ratio values in the ratio image and saves a table of ratios and corresponding ion concentrations. The table will be saved as a *.gry file, which can then be used in MetaMorph to apply the calibration using the Calibrate Gray Levels command (Measure menu) for measurement of brightness, optical density, etc.

Cancel

Cancels the command.

Import Image-1/FL CAL File (Calibration Menu)

Imports Image-1/FL equation calibration files.

Use this command when you want to read in an equation calibration file to MetaFluor that was generated with Image-1/FL.

Note: You will not be able to import Image-1/FL *titration* calibration files.

See Also:

Equation Calibration

Importing an Image-1/FL CAL File

To import an Image-1/FL .cal file, use the following procedure:

Step	Action
1	From the Calibration menu, choose Import Image-1/FL CAL File. The Select Image-1/FL CAL File dialog box will appear.
2	Select the icon for the desired file, using the *Look In* drop-down list box or Up One Level icon button to locate the file if necessary. Then choose *Open.*
3	After the file has been read, a message box will appear which confirms that the Image-1/FL .cal file has been imported and the equation constants have been calculated. Choose *OK.*
4	The Equation Calibration in vitro dialog box will appear with the equation variables filled in for the appropriate text boxes.
5	Type the appropriate *Kd Value* and *Viscosity* constants in the *Equation Constants* text boxes. Continue by following the procedure for the use of the **Equation Calibration in vitro** command.

Import Image-1/FL CAL File - Dialog Box Options

File Name

Lists the name of the currently selected file.

Files of Type

Determines the file format of the files displayed in the *File Name* list.

Look In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level icon button to go up one level in the directory structure.

Open

Opens the Image-1/FL .cal file.

Cancel

Cancels the command.

Journals Menu

Create Journal (Journals Menu)

Creates and saves a new journal using the Journal Editor.

Use this command to create new MetaFluor journals. The Journal Editor enables you to select the functions that you want to included in a journal.

The Journal Editor dialog box contains two tabbed areas. The Functions area on the left side of the dialog box contains all of the available functions that initiate specific commands. You can display this list in either alphabetically or according to the functions associated with each menu and command. The *Journal* area on the right lists the functions that have been added to the currently selected journal, in the order that they will occur.

The Edit Journal command enables you to select any journal for editing. However, if you want to edit a journal that is part of the current taskbar,. simply press and hold the [SHIFT] key while you click the associated button on the taskbar. This opens the Journal Editor with the selected journal ready for editing.

After you have created a collection of journals, you can assign them all to the same **journal toolbar** for ease of use.

When you create journals, you should remember that shorter journals are easier to manage and easier to troubleshoot (if necessary).

Notes:

❑ If you load an updated version of MetaFluor to a different directory from the previous version, you will need to update your old journals with the Edit Journal command so that the journals will be configured to look in the appropriate directories.

❑ The Command *"***End of Journal***"* indicates the end of the journal. This is the last command in every journal and is a required command. You cannot delete this command.

Shortcut: CTRL + N

See Also:

Create Journal Toolbar

Edit Journal

List of Journal Functions

Creating a Journal

To create a journal, use the following procedure:

Step	Action
1	From the Journals menu, choose Create Journal. The Select a New Journal Name dialog box will appear.
2	Type a name for the new journal in the *File Name* text box. MetaFluor will assign the file extension ".jnl" to your file name. **AND** Choose *Save.* The Journal Editor dialog box will open.
3	From the *Builtin Functions* tab in the *View* box, select the view that you want to use to display the list of journal functions: *Alphabetical* or *Task.*
4	Highlight the function that you want to add to the journal *Function List.*
5	Choose *Copy,* or drag the function from the Function List to the list of journal entries on the right. You can add entries to your journal by dragging functions to either the Functions or Descriptions box on the right or by using another appropriate editing method, such as Copy and Paste*.*
6	When you select a function on either the functions tab or the descriptions tab, the lower portion of the tab shows you available parameters and options that you can set. *Playback Interactively* will change an entry's interactive status. If there is an "I" displayed in front of the entry, the journal will stop during playback to display a dialog box, so that you can modify the function's parameters (if there are any parameters that can be edited). This command is the same as the Toggle Interactive command in the Journal menu.
7	To save a journal after editing, choose *File>Save.* This will overwrite the existing version of the journal. **OR** Choose *File>Save As* to save the edited journal under a new name so that the original file remains intact. Type the file name in the *File Name* text box in the Save Journal As dialog box and choose *Save.*
8	To print your completed journal, choose *File>Print.* The entire journal is sent to the

currently selected Windows printer. Icons are not shown in the printed version, only text.

9   Use the *File>Close* command to close any journals you do not want to use. (Remember to save first!)

10   When you have finished editing and saving your journals, choose *File>Exit* to close the Journal Editor dialog box.

Create Journal - Dialog Box Options

File

Provides a menu that contains a set of commands to enable you to originate new journals and to edit, save and print existing journals.

New

Creates a new journal file.

Open

Opens the selected journal file.

Close

Closes the active journal.

Save

Saves the current journal, overwriting the contents of the journal file if it has been previously saved.

Save As

Saves the current journal using a different file name of your choice.

Revert to Saved

Restores the currently displayed journal to the condition it was in when it was last saved.

Print

Opens the Windows Print dialog box and enables you to print a copy of the journal to the selected Windows device. Icons are not shown in the printed version, only text.

Exit

Discontinues any running journal and closes the Journal Editor dialog box.

Edit

Provides a menu that contains commands for cutting, copying, pasting, and controlling journal functions.

Cut

Deletes the selected function from the current journal.

Copy

Copies the selected function from the current journal.

Paste

Pastes the most recently cut or copied function to the current journal, placing it above the currently selected function in the list of functions.

Delete

Permanently removes the selected journal function.

Disable

Temporarily deactivates the selected journal function.

Interactive

Turns on or off (toggles) the interactive journal mode. A check next to this setting indicates that it is active (on).

Override Settings

Enables you to temporarily override the current settings and replace them with new settings.

Built-in Functions

Lists functions that can be added to a journal. Functions can be added by double-clicking a function name in the table, or by dragging a function from the table to the list of functions in the current journal.

View

Selects a view for the display of the journal functions in the Function Table: Alphabetical or by Menu.

Recorded Journals

Shows your currently accessed folder and associated path. Use this tab to move your folder selection from one folder to another. From the appropriate journal folder, double-click the name of the journal that you want to open for viewing or editing, or to run. You can also double-click or drag journals from these folders into the currently open journal to run journals from within a journal or to loop a journal.

Actions

Shows a list of programming commands that you can include in your journal. Double-click the name of the command or drag the command into the appropriate location in your journal

Built-in Functions

Lists functions that can be added to a journal. Functions can be added by double-clicking a function name in the table, or by dragging a function from the table to the list of functions in the current journal.

Journal

Lists the active journals that are open for editing. The journal name displayed is the current journal. The status text next this option lists how many journals are open for editing.

Functions

Lists all of the functions in the current journal. Click an entry once to select it for cutting, copying, pasting, editing, or toggling the interactive mode. Double-click an entry to edit the entry. Select the entry and press Delete to remove the entry, or right click and select Delete. Only the function names are shown in this window. Choose the Description tab to see any variables or parameters assigned to the function or action. This table is located on the right side of the dialog box.

Descriptions

Shows the same information as the Functions tab, but also includes any assigned variables and parameter settings.

For both the *Functions* tab and the *Descriptions* tab, the following selections are available for most functions. Selected programming actions will show entry boxes for all applicable parameters, variables and settings for the programming action.

Playback Interactively

Enables interactive journal editing. With interactive journal editing, you can modify function settings during journal playback. For any functions that have modifiable settings, the journal will pause and open a dialog box for each function for which you have checked *Playback Interactively*.

Disable

Deactivates the selected function without removing it from the journal or changing any of the function settings.

Edit Function Settings

Opens the associated settings dialog box for the selected function.

Select Settings to Override

For specific function settings in specific function dialog boxes, enables you to temporarily select and override certain settings with new values.

Undo

Resets all settings for the function to the previously set values.

Edit Journal (Journals Menu)

Edits a journal that was created previously and saved.

Use this command when you want to make changes to a journal. After you select the journal you want to edit, this command will open the Journal Editor so that it can make the necessary changes. Use this command to add journal entries or to edit the parameters for a journal entry. Also use this command to add programming actions that cannot be created in a journal by recording the journal.

The Journal Editor dialog box contains two tabbed areas. The Functions area on the left side of the dialog box contains all of the available functions that initiate specific commands. You can display this list in either alphabetically or according to the functions associated with each menu and command. The *Journal* area on the right lists the functions that have been added to the currently selected journal, in the order that they will occur.

Interactive Mode stops the journal during playback so that you can modify its parameters. This option can be selected when you add a new function to a journal. You can also change a particular journal entry's interactive mode status by highlighting the desired entry in the list on the right and choose *Toggle Interactive.* This will toggle the entry's status to on ("X") or off (no "X"), depending on its current state.

Using the Journal Editor dialog box, you can edit several journals at once without returning to the Journals menu. You can cut, copy, and paste journal entries to and from the various journals as you are editing them.

If you want to edit a journal that is part of the current journal toolbar, there is a quick way to select it without using the Edit Journal command. Simply press and hold the [SHIFT] key while clicking the journal's Toolbar button. This will open the Journal Editor with the selected journal ready for editing.

Note: If you load an updated version of MetaFluor to a different directory from the previous version, you will need to update your old journals with the Edit Journal command so that the journals will be configured to look in the appropriate directories.

See Also:

Create Journal

Create Journal Toolbar

List of Journal Functions

Editing a Journal

To edit one or more journals, use the following procedure:

Step	Action
1	From the Journal menu, choose Edit Journal. The *Select a Journal to Edit* dialog box opens.
2	Select the journal that you want to edit. If necessary, use the *Look In* list or Up One Level button to locate the correct drive and folder. Then choose *Open.* The Journal Editor dialog box will appear.
3	If you want to edit more than one previously saved journal at once, choose *File>Open.* The Open a Journal to Edit dialog box will open. Select the file for the desired journal and choose *Open.* (You can open previously saved journals at any time while editing a journal.)
4	The last journal open will become the current journal, as listed in the drop-down list in the upper right corner of the dialog box. You can edit another open journal by selecting it from the drop-down list or, if the taskbar to which it has been assigned is currently displayed, you can "shift-click" its assigned button (hold down the [SHIFT] key and click the taskbar button using your left mouse button). The status text at the top of the dialog box will note how many journals are currently open.
5	From the *View* list, select the view you want to use; either *Alphabetical* or *Menu.*
6	You can add entries to your journal by dragging functions to either the Functions or Descriptions box on the right or by using another appropriate editing method, such as Copy and Paste.
7	*Playback Interactively* will change an entry's interactive status. If there is an "I" displayed in front of the entry, the journal will stop during playback to display a dialog box, so that you can modify the function's parameters (if there are any parameters that can be edited). This command is the same as the Toggle Interactive command in the Journal menu.
8	To save a journal after editing, choose *File>Save.* This will overwrite the existing version of the journal. **OR** Choose *File>Save As* to save the edited

journal under a new name so that the original file remains intact. Type the file name in the *File Name* text box in the Save Journal As dialog box and choose *Save.*

9   To print your completed journal, choose *File>Print.* The entire journal is sent to the currently selected Windows printer. Icons are not shown in the printed version, only text.

10   You can use the *File>Close* command to close any journals you do not want to use. (Remember to save first!)

11   When you have finished editing and saving your journals, choose *File>Exit* to close the Journal Editor dialog box.

Adding a Journal Entry

To add a journal entry to the journal you are editing, use the following procedure:

Step	Action
1	From the *View* list, select the view you want to use to display the Function List: either *Alphabetical* or *Menu.*
2	From the Built-in Functions list on the left, select the function that you want to add to your journal. **AND** Choose *Edit>Copy* or drag the function from the Built-in Functions List to the list of journal entries on the right.
3	If the command has parameters or settings that you can edit (such as an enabled/disabled state), its dialog box will appear so that you can change its options. Choose *OK* when you have finished. If there are no parameters or options to set, a dialog box with the message "This journal entry is not editable" will appear.
4	The function will now appear in the right column before the previously selected entry. If you selected *Playback Interactively, a*n "I" will appear before the function name in both the *Functions* tab and the *Descriptions* tab.

Cutting, Copying, and Pasting Journal Entries

If you want to remove the currently highlighted entry from your journal list, choose *Edit>Cut,* OR right-click and choose *Cut*, OR Ctrl+X, OR Press the Delete key.

If you want to copy the currently highlighted entry from your journal list, choose *Edit>Copy,* OR right-click and choose *Copy*, OR Ctrl+C.

If you want to paste the last entry that was cut or copied, highlight the entry and choose *Edit>Paste*, OR right-click and choose *Paste*, OR Ctrl+V. The pasted entry will appear above the currently highlighted entry.

You can cut and copy multiple entries from the journal. To select a set of adjacent entries, select the first entry and then hold down the [SHIFT] key while selecting the last entry in the list. All entries in between the two selected entries will also be selected. To select multiple entries scattered throughout the list, select the first entry, and then hold down the [CTRL] key while selecting the other desired entries. Only these entries will be highlighted. You can cut and copy as described above.

Editing Entries in a Journal

As you edit a journal, you can decide to change the parameters or settings that you selected for a particular function. To change the parameters or settings, highlight the desired entry in the list on the right and click *Edit Function Setting.* You will then be able to change the function's editable parameters in its dialog box. Choose *OK, Apply,* or *Record* (as applicable) to record the changes in the function's parameters.

If you want to change a particular journal entry's interactive mode status, highlight the desired entry in the right-hand list and choose *Play Interactively.* This will toggle the entry's status to on ("I") or off (no "I") depending on its current state.

Note: When you change the setting in a command's dialog box while editing a journal, you **must** choose its *OK, Apply or Record,* or other applicable command button for your changes to be recorded.

Edit Journal - Dialog Box Options

File

Provides a menu that contains a set of commands to enable you to originate new journals and to edit, save and print existing journals.

New

Creates a new journal file.

Open

Opens the selected journal file.

Close

Closes the active journal.

Save

Saves the current journal, overwriting the contents of the journal file if it has been previously saved.

Save As

Saves the current journal using a different file name of your choice.

Revert to Saved

Restores the currently displayed journal to the condition it was in when it was last saved.

Print

Opens the Windows Print dialog box and enables you to print a copy of the journal to the selected Windows device. Icons are not shown in the printed version, only text.

Exit

Discontinues any running journal and closes the Journal Editor dialog box.

Edit

Provides a menu that contains commands for cutting, copying, pasting, and controlling journal functions.

Cut

Deletes the selected function from the current journal.

Copy

Copies the selected function from the current journal.

Paste

Pastes the most recently cut or copied function to the current journal, placing it above the currently selected function in the list of functions.

Delete

Permanently removes the selected journal function.

Disable

Temporarily deactivates the selected journal function.

Interactive

Turns on or off (toggles) the interactive journal mode. A check next to this setting indicates that it is active (on).

Override Settings

Enables you to temporarily override the current settings and replace them with new settings.

Built-in Functions

Lists functions that can be added to a journal. Functions can be added by double-clicking a function name in the table, or by dragging a function from the table to the list of functions in the current journal.

View

Selects a view for the display of the journal functions in the Function Table: Alphabetical or by Menu.

Recorded Journals

Shows your currently accessed folder and associated path. Use this tab to move your folder selection from one folder to another. From the appropriate journal folder, double-click the name of the journal that you want to open for viewing or editing, or to run. You can also double-click or drag journals from these folders into the currently open journal to run journals from within a journal or to loop a journal.

Actions

Shows a list of programming commands that you can include in your journal. Double-click the name of the command or drag the command into the appropriate location in your journal

Built-in Functions

Lists functions that can be added to a journal. Functions can be added by double-clicking a function name in the table, or by dragging a function from the table to the list of functions in the current journal.

Journal

Lists the active journals that are open for editing. The journal name displayed is the current journal. The status text next this option lists how many journals are open for editing.

Functions

Lists all of the functions in the current journal. Click an entry once to select it for cutting, copying, pasting, editing, or toggling the interactive mode. Double-click an entry to edit the entry. Select the entry and press Delete to remove the entry, or right click and select Delete. Only the function names are shown in this window. Choose the Description tab to see any variables or parameters assigned to the function or action. This table is located on the right side of the dialog box.

Descriptions

Shows the same information as the Functions tab, but also includes any assigned variables and parameter settings.

For both the *Functions* tab and the *Descriptions* tab, the following selections are available for most functions. Selected programming actions will show entry boxes for all applicable parameters, variables and settings for the programming action.

Playback Interactively

Enables interactive journal editing. With interactive journal editing, you can modify function settings during journal playback. For any functions that have modifiable settings, the journal will pause and open a dialog box for each function for which you have checked *Playback Interactively*.

Disable

Deactivates the selected function without removing it from the journal or changing any of the function settings.

Edit Function Settings

Opens the associated settings dialog box for the selected function.

Select Settings to Override

For specific function settings in specific function dialog boxes, enables you to temporarily select and override certain settings with new values.

Undo

Resets all settings for the function to the previously set values.

Save

Saves the current journal, overwriting the contents of the journal file if it has been previously saved.

Run Journal

Runs the current open journal. The journal must be saved before it will run.

Exit

Closes any running journal and closes the Journal Editor dialog box.

Run Journal (Journals Menu)

Allows you to select a previously saved journal and run it at that particular moment.

This command is useful for running journals that you do not use frequently and which are not used in conjunction with other journals.

For journals that you use frequently, it is best to assign them to a journal toolbar with other similar journals, and run them directly from the toolbar as needed, rather than using the Run Journal command. If you need to run a journal after a specific event (for example, after each acquisition cycle), you should use the Auto-Execute Journals command.

WARNING:
When you use journals during a procedure that involves an illumination device, you should close the Illumination Control dialog box before starting the journal. Attempts to run a journal while the Illumination Control dialog box is still open may cause your system to appear to "freeze." This is due to the "modal" nature of dialog boxes that are opened in journal playback mode, combined with the Illumination Control dialog box's need to reconfigure itself by closing itself and reopening when a new illumination device is selected.

See Also:

Create Journal

Create Journal Toolbar

Auto-Execute Journals

Configure Illumination

Running a Journal

To run a journal, use the following procedure:

Step	Action
1	From the Journals menu, choose Run Journal. The Select a Journal to Run dialog box will appear.
2	Select the desired journal. If necessary, use the *Look In* list or Up One Level button to locate the appropriate drive and folder.
3	Choose *Open.* The dialog box will close and the journal will run.

Select a Journal to Run - Dialog Box Options

File Name

Lists the name of the currently selected journal file.

Files of Type

Determines the format of the files displayed in the *File Name* list.

Look In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

Open

Opens the file.

Cancel

Cancels the command.

Auto-Execute Journals (Journals Menu)

Allows you to run previously created and saved journals automatically.

Use this command to run previously created and saved journals automatically when selected events occur. The Auto-Execute Journals dialog box has a list of events for which you can specify an autoexecuting journal. You can associate a journal with the start or end of a new experiment, the opening or closing of a stored experiment, the moment before, during, or after acquisition of a wavelength image, or upon termination of acquisition. In the Auto-Execute Journals dialog box, you can select only one journal per event. However, there is a command available in the Journal Editor for executing a journal while running another one, so it is possible to run more than one journal per event. You can save sets of associations between events and journals in an autoexecute journal list file (*.aej) and load saved files from disk. If an error occurs while the journal is running, a message box will appear, asking if you want to remove the association between the offending journal and the event.

Note: This command is unavailable in the MetaFluor Offline system.

Note: MetaFluor will autoexecute journals only if the **Auto-Execute Journals** command in the Journals menu has been enabled (a check mark will appear next to its name). This is so that you can disable the Auto-Execute Journal command if you need to start or open an experiment without the journals running.

See Also:

Use Auto-Execute Journals

Autoexecuting Journals

To autoexecute journals, use the following procedure:

Step	Action
1	From the Journals menu, choose Auto-Execute Journals. The Auto-Execute Journals dialog box will appear.
2	If you have previously saved an autoexecute journals list file and want to use it, choose *Load List.* The Select an AutoExecute Journal List dialog box will appear. Select the desired file and choose *Open.* Then skip to Step 7.
	OR
	If you want to create a new set of event/journal associations, continue to Step 3.
3	To assign a journal to a specific task/event, highlight the task in the *Tasks Which Can Execute Journals* table and choose *Assign Journal.* Alternatively, you can double-click the task's entry in the table.
4	The Select a Journal dialog box will appear. Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button to locate the appropriate drive and folder. Then choose *Open.*
	A check mark will appear in front of the task name, and the status line at the bottom of the dialog box will indicate the name of the journal file associated with the task. If you need to remove a journal assignment, highlight the pertinent task and choose *Clear Journal.*
5	Repeat Steps 3 and 4 for each journal association you want to assign.
6	If you want to save the set of event/journal associations, choose *Save List.* The Save AutoExecute Journal List dialog box will appear.
	AND
	Type a name for the autoexecute journal list file in the *File Name* text box and choose *Save.*
7	Choose *Close* when you have finished.

Auto-Execute Journals - Dialog Box Options

Tasks Which Can Execute Journals

Lists the tasks or events which you can assign a journal to autoexecute when that event occurs. A check mark will appear next to the events that have a journal associated with them. The status text below this list box displays the name of the journal that will be autoexecuted when the selected task or event occurs.

Load List

Opens the Select an AutoExecute Journal List dialog box, from which you can load a previously saved set of task/journal associations.

Save List

Opens the Save AutoExecute Journal List dialog box, with which you can save a set of task/journal associations in an autoexecute journals list file (*.aej).

Assign Journal

Assigns a journal to the selected task using the Select a Journal dialog box.

Clear Journal

Clears the journal associated with the selected task/event. Displays "<None>" to indicate that there is no journal associated with the selected task or event.

Clear All Journals

Clears all journals from all tasks.

Close

Closes the dialog box.

Use Auto-Execute Journals (Journals Menu)

Enables and disables the Auto-Execute Journals command.

This command acts as a toggle, enabling or disabling the **Auto-Execute Journals** command. After you have selected the journals to autoexecute using the Auto-Execute Journals command, you will need to enable the Use Auto-Execute Journals command by choosing it from the Journals menu, so that a check mark appears before its name.

If you do not want to run the journals at any point before or during an experiment, choose the Use Auto-Execute Journals command again from the Journals menu. This will disable the Auto-Execute Journals command, and the check mark in the Use Auto-Execute Journals menu will disappear.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Auto-Execute Journals

Using the Auto-Execute Journals Command

To enable the Use Auto-Execute Journals command, use the following procedure:

Step	Action
1	Select the Journals menu.
2	Choose Use Auto-Execute Journals. This will toggle the command's state. If it is enabled, a check mark will appear next to its name on the menu.
	If the command is inactive, choosing Use Auto-Execute Journals will enable it and a check mark will appear next to its name. Choosing the command again will disable it and remove the check mark.

Sequence Journals (Journals Menu)

Sets up a sequence of journals that can be run at a specified time in the experiment or after a specified number of acquisition cycles have occurred.

Use this command to create a sequence of journals defined by time or cycle number. You can select a specific time or acquisition cycle number in the experiment, or select a time or acquisition cycle within a configured sequence. The journals must already exist before you create the sequence. There is no limit to the number of journals that can be contained in a sequence.

Note: This command is unavailable in the MetaFluor Offline system.

You can also use this command to save and load a sequence list and to edit an existing sequence list. If necessary, you can reset the cycle count prior to starting the sequence. Likewise, you can zero the clock prior to the sequence. The current time, cycle number, and last journal run will be indicated in a Sequence Status window, which you can choose whether or not to display.

After you have set up the sequence, you can use the **Use Sequence Journals** command to enable or disable sequencing during image acquisition. Or you can use the **Run Sequence** command to start acquisition and initiate the sequence.

The journals in the sequence will run when their times or cycle numbers are reached in the experiment. For instance, you can set up a sequence to run a journal when an experiment is started, to run another journal one minute later, and run a third journal five minutes after that. This command does not repeat the journal sequence.

If you need to rezero the sequence clock after starting a journal sequence, you will need to have added the Zero Sequence Clock journal function to the journal before you start the sequence. The only other way you will be able to restart the sequence timer is to close the Sequence Status window and choose Run Sequence again from the Journal menu. If you merely want to stop the sequence cycle, you can close the Experiment Control Panel, but the timer will continue to run.

See Also:

Use Sequence Journals

Run Sequence

Sequencing Journals

To sequence journals, use the following procedure:

Step	Action
1	From the Journal menu, choose Sequence Journals. The Sequence Journals dialog box will appear.
2	To add a journal to the sequence, choose *Add.* The Add Sequence Journal dialog box will appear.
3	Choose *Select Journal.* The Select a Journal dialog box will appear.
	AND
	Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button to locate the appropriate drive and folder. Then choose *Open* to return to the Add Sequence Journal dialog box.
4	Select:
	Execute at Experiment Time if you want the journal to run at a specific time in the experiment. Use its spin box and drop-down list to set the amount of time and units of measure.
	Execute on Experiment Cycle if you want the journal to run at a specific acquisition cycle, counted from the beginning of the experimental acquisition. Use its spin box to select the acquisition cycle number.
	Execute at Sequence Time if you want the journal to run at a specific time from the start of the sequence timer. Use its spin box and drop-down list to set the amount of time and units of measure.
	Execute on Sequence Count if you want the journal to run at a specific acquisition cycle after you start the sequence timer. Use its spin box to select the acquisition cycle number.
5	When you have finished, choose *OK.* The Sequence Journals dialog box will reappear.
6	Repeat Steps 2 - 5 for each journal you want to add.
	When you have finished adding journals, verify that all of the journals that you want to use have check marks in front of their names in the *Journal Sequence List.* If not, double-click the name to toggle the check mark on.
7	If you want to zero the clock prior to the sequence when using the Run Sequence command, select *Zero the Experiment Clock.*

Likewise, select *Zero the Experiment Cycle Counter* if you want to reset the cycle prior to the sequence when using the Run Sequence command.

8   If you do not want to see the Sequence Status window when using the Run Sequence command, clear the *Display the Sequence Status Window* check box.

OR

If you want to be able to see the current time, cycle count, and last journal run while using the Run Sequence command, leave the *Display the Sequence Status Window* check box selected (the default state).

9   Choose *OK* when you have finished.

Sequence Journals - Dialog Box Options

Journal Sequence List

Lists the journals in sequence and displays when each will be run. Those indicated with a check mark will be run during the sequence. To enable or disable the check mark, double-click the journal name.

Add

Adds a journal to the sequence list. This command opens the ***Add Sequence Journal*** dialog box. Use *Select Journal* to select the journal you want to add. You can specify that the journal be run after a specific amount of time after image acquisition is started, or you can specify that the journal be run after a specific number of cycles has occurred.

Remove

Removes the selected journal from the *Journal Sequence List.*

Edit

Allows you to change the journal that is run, and when it is to be run. This command opens the Edit Sequence dialog box. Use *Select Journal* to change the journal. You can specify that the journal be run after a specific amount of time after image acquisition is started, and you can specify that the journal be run after a specific number of cycles has occurred.

Save List

Saves the current sequence list. This command opens the Save Journal Sequence dialog box. Use its *Sequence List File* command to specify the file name for saving the list. Choose *OK* when you have finished.

Load List

Loads a previously saved sequence list. This command opens the Load Journal Sequence dialog box. Use its *Select a Sequence List* to specify the file name of the list you want to load. Choose *OK* when you have finished.

Zero the Experiment Clock

Resets the clock counter to zero prior to starting a sequence with the Run Sequence command.

Zero the Experiment Cycle Counter

Resets the cycle count prior to starting a sequence with the Run Sequence command.

Display the Sequence Status Window

Displays the Sequence Status window when you run the Run Sequence command. This window indicates the current time, cycle count, and the last journal run.

OK

Configures the sequence of journals.

Add Sequence Journals - Dialog Box Options

Select Journal

Displays the Select a Journal dialog box, from which you can select the journal for which you want to configure the sequence timing.

Execute at Experiment Time

Selects a specific time in the experiment for the selected journal to run.

Execute on Experiment Cycle

Selects a acquisition cycle in the experiment for the selected journal to run.

Execute at Sequence Time

Selects a specific time from the start of the sequence timer for a journal to run.

Execute on Sequence Count

Selects a specific acquisition cycle after you start the sequence timer for a journal to run.

OK

Accepts the timing configuration for the selected journal and returns you to the Sequence Journals dialog box.

Cancel

Cancels the command and returns you to the Sequence Journals dialog box.

Use Sequence Journals (Journals Menu)

Enables or disables the use of the Sequence Journals command.

Use this command to enable or disable the journal sequencing that was set up in the
Sequence Journals dialog box. If you start image acquisition with this command enabled, it will
start the sequence that you have set up in the Sequence Journals dialog box. If this command
is not already enabled when you use the **Run Sequence** command, it will be enabled at that
time.

You must set up the sequence using the **Sequence Journals** command prior to using this
command.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Sequence Journals

Run Sequence

Using Sequence Journals

To enable the Use Sequence Journals command, use the following procedure:

Step	Action
1	Select the Journals menu.
2	Choose Use Sequence Journals. This will toggle the command's state. If it is enabled, a check mark will appear before its name on the menu. If it is enabled, choosing Use Sequence Journals will disable it and the check mark will be removed from the menu.

Run Sequence (Journals Menu)

Starts image acquisition and runs a sequence of journals at a specified time in the experiment or after a specified number of cycles have occurred.

Use this command to start image acquisition and run a sequence of journals defined by time or cycle number. The journals must already exist before you create the sequence. If you selected *Zero the Experiment Cycle Counter* in the Sequence Journals dialog box, the cycle count will be reset prior to starting the sequence. Likewise, if you selected *Zero the Experiment Clock,* the clock will be zeroed prior to the sequence. If you selected *Display the Sequence Status Window,* the Sequence Status window will be displayed, providing you with the current time, cycle count, and the last journal run.

You must set up the sequence using the **Sequence Journals** command prior to using this command.

This command automatically enables the **Use Sequence Journals** command if it is not already enabled.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Sequence Journals

Use Sequence Journals

Running a Journal Sequence

To run a journal sequence, use the following procedure:

Step	Action
1	Select the Journals menu.
2	Choose Run Sequence. If the Use Sequence Journals command is not enabled, Run Sequence will be disabled.
3	If you selected *Zero the Experiment Cycle Counter* or *Zero the Experiment Clock* in the Sequence Journals dialog box, the counter(s) will be reset. If you selected *Display the Sequence Status Window* in the Sequence Journals dialog box, the Sequence Status window will appear, indicating the current time, cycle count, and the last journal run.
4	The Run Sequence command will then start image acquisition and run the journal sequence.
5	If an error occurs while the journal is running, a message box will appear, asking if you want to remove the offending journal.

Trigger Journals (Journals Menu)

Configures the trigger input devices and trigger conditions for Use Trigger Journal and Wait for Trigger commands.

Use this command to add or edit a trigger input device driver. This command allows you to specify the device used and its configuration.

After you have configured the driver, you can create a trigger condition that will instruct MetaFluor to run a particular journal when the computer receives a signal from the specified trigger input device that meets the condition. Each condition includes (1) the trigger input device supplying the signal, (2) the voltage signal pin, and (3) the desired voltage signal state (high or low) that must be received from the trigger input device to run the specified journal. You can create a list of conditions and enable them as you need them. MetaFluor will wait for only those conditions that are selected in the Trigger Journals dialog box.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Use Trigger Journal

Wait for Trigger

Configuring Trigger Journals

To configure trigger journals, use the following procedure:

Step	Action
1	From the Journals menu, choose Trigger Journals. The Trigger Journals dialog box will appear.
2	To add and configure a new trigger input device, choose *Add.* The Open DIO Driver dialog box will appear.
3	Type the name of the device in the *Device Name* text box.
4	Use the *DIO Hardware* drop-down list to specify the type of digital I/O device hardware you are using.
5	Choose *Change Driver Configuration.* The Digital I/O Configuration dialog box will appear. The options displayed in this dialog will depend on the type of hardware you selected in Step 4. Select the appropriate settings and choose *OK.*
6	Choose *OK* to complete the configuration of the trigger input device and return to the Trigger Journals dialog box.
7	To add a new trigger condition, choose *Add.* The Add Trigger Condition dialog box will appear.
8	Choose *Select Journal.* The Select a Journal dialog box will appear. Select the icon for the desired journal. Use the *Look In* list or Up One Level icon button if necessary to locate the journal file. Then choose *Open.*
9	Select the desired trigger input device from *Trigger Input Device* drop-down list. Use *Trigger Input Pin* # to select the input pin that will be receiving the signal. Then use *Trigger When Pin Goes* to select the signal *(High* or *Low)* that will trigger the playback of the associated journal. Choose *OK.*
10	Repeat Steps 7 - 9 for all of the trigger conditions you want to set up. Then double-click each one you want to use, to enable it (a check mark will appear in front of all of the conditions that are enabled).
11	Choose *OK* when you have finished.

Trigger Journals - Dialog Box Options

Trigger Input Devices

Lists the trigger input devices that have been configured.

Add (Trigger Input Devices)

Adds and configures a new trigger input device. Use *Device Name* to specify a unique name for the device. Use *DIO Hardware* to specify the hardware you are using for the trigger device. *Change Driver Configuration* allows you to set the base address and data flow direction for the device (or to specify the parallel port and IRQ setting if you are using a parallel port).

Remove (Trigger Input Devices)

Removes the selected trigger input device from the *Trigger Input Devices* list.

Edit (Trigger Input Devices)

Allows you to edit a previously configured trigger input device. The options available are the same as those for the *Add* command. You can also edit a trigger input device by double-clicking its name in the *Trigger Input Devices* list.

Trigger Conditions

Lists the trigger conditions that have been defined. Only those that are enabled will be used by the trigger commands. To enable a trigger condition, double-click its name so that a check mark appears in front of the name.

Add (Trigger Conditions)

Adds and configures a trigger condition.

Select Journal selects a journal to be associated with the trigger. After you select a journal, its name will be listed in the Add Trigger Conditions dialog box.

Trigger Input Device selects a trigger input device to use for the condition.

Trigger Input Pin # specifies the pin number that will receive the signal.

Trigger When Pin Goes (High or *Low)* specifies the desired state of the pin necessary to trigger the journal.

Remove (Trigger Conditions)

Removes the selected trigger condition from the *Trigger Conditions* list.

Edit (Trigger Conditions)

Allows you to edit a previously configured trigger input device. The options available are the same as those for the Add command.

OK

Closes the Trigger Journals dialog box.

Use Trigger Journals (Journals Menu)

Enables or disables the use of the Trigger Journals command.

Use this command to enable or disable the use of the trigger conditions set up in the Trigger Conditions dialog box. When enabled (marked with a check mark), this command polls the trigger input device to see if a trigger condition has been met; if it has, this command runs the journal specified in the condition.

Use the **Trigger Journals** command to set up the condition(s) prior to using this command.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Trigger Journals

Wait for Trigger

Using Trigger Journals

To enable the Use Trigger Journals command, use the following procedure:

Step	Action
1	Select the Journals menu.
2	Choose Use Trigger Journals. This will toggle the command's state. If it is enabled, a check mark will appear before its name on the menu. If it is enabled, choosing Use Trigger Journals will disable it and the check mark will be removed from the menu.

Wait for Trigger (Journals Menu)

Waits for the selected trigger condition(s) to be met and runs the associated journal as each condition is met.

Use this command when you want MetaFluor to suspend all activity until a signal that meets the selected trigger condition(s) is received from the trigger input device. When a condition is met, its journal will be run. Because the Wait for Trigger dialog box is modal (operates in a restricted mode), you will not be able to use any other MetaFluor command until all of the conditions are met or until you cancel the command.

This command waits for those conditions that were enabled in the Trigger Journals dialog box.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Trigger Journals

Use Trigger Journals

Waiting for a Trigger

To configure MetaFluor to wait for a trigger, use the following procedure:

Step	Action
1	From the Journals menu, choose Wait for Trigger. The Wait for Trigger dialog box will appear.
2	When the conditions specified in the Trigger Journals dialog box have been met, the Wait for Trigger dialog box will close. If an error occurs while the journal is running, a message box will appear, asking if you want to remove the offending journal.
3	If you want to cancel the Wait for Trigger command before the remaining condition(s) are met, choose *Cancel.*

Create Journal Toolbar (Journals Menu)

Creates a toolbar consisting of buttons that allow you to run a journal with a single click of a mouse button.

Use this command to create toolbars for accessing frequently used groups of related journals. You can create and save as many journal toolbars as you want, but only one can be used at a time. Each journal toolbar can consist of up to 50 buttons in a configuration of rows and columns of your choice. By adjusting the width of the buttons, you can create toolbars with very small buttons that resemble an icon bar, or you can create toolbars with long buttons for descriptive text. You can also embellish the buttons with a button graphic, such as a small colored square or an arrowhead.

After a toolbar is created, you can immediately assign previously created journals to its buttons. Use the Edit Journal Toolbar command to assign journals to buttons later.

Note: If you load an updated version of MetaFluor to a different directory from the previous version, you will need to update your old journal toolbars with the Edit Journal Toolbar command so that the toolbars will be configured to look for its journals in the appropriate directory.

See Also:

Edit Journal Toolbar

Load Journal Toolbar

List of Journal Functions

Creating a Journal Toolbar

To create a journal toolbar, use the following procedure:

Step	Action
1	From the Journals menu, choose Create Journal Toolbar. The Create Journal Toolbar dialog box will appear.
2	Select the number of rows and columns for the toolbar using *Rows* and *Columns.* Then select the *Width (# Letters) of Each Button.* Choose *OK.*
3	If desired, type a title for the Toolbar in the *Journal Toolbar Title* text box.
4	Choose *OK.* The Create Journal Toolbar dialog box will close, and the Select a New Journal Toolbar Name dialog box will appear.
	Type the file name for the toolbar in the *File Name* text box. The file extension ".jtb" will be assigned to your file name. Choose *Save.*
5	The Edit Journal Toolbar dialog box will appear. This consists of two tabbed pages, a Buttons page which you will use to assign functions to the Toolbar buttons, and a Toolbar page which consists of the options you used in Steps 2 and 3.
	Select the Buttons tab.
6	To assign a journal to a button, highlight the desired button location for the journal name in the *Journal Name* list and choose *Assign Entry.*
7	The Select a Journal dialog box will appear. Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button to locate the appropriate drive and folder. Then choose *Open.*
8	When the journal name appears in the *Journal Name* table, type the text that is to appear on the button in the *Wording to Appear on Button* text box.
9	If you want to place a graphic on the button (a colored square or a directional arrowhead), choose the *Button Graphic* command button. The Button Graphic dialog box will appear.
	Click the graphic of your choice. The Button Graphic dialog box will close automatically. and the graphic you chose will appear on the *Button Graphic* command button for the selected entry in the *Journal Name* table.
10	Repeat Steps 6 - 9 until you have assigned a journal to each button. If you wish, you can

leave some buttons blank for future use.

11 When you have finished, choose *Close*.

Create Journal Toolbar - Dialog Box Options

Rows

Specifies the number of rows for the journal toolbar.

Columns

Specifies the number of columns for the journal toolbar.

Width (# Letters) of Each Button

Specifies the width of each button (number of letters that appears on the button).

Journal Toolbar Title

Specifies the title that appears at the top of the toolbar.

OK

Creates the toolbar and opens the Select a New Journal Toolbar Name dialog box to save the toolbar file. Type the desired file name in the *File Name* text box and choose *Save.*

Cancel

Cancels the command.

Edit Journal Toolbar - Dialog Box Options

Buttons Tab Page

Journal Name

Lists the names of the journals assigned to each button in the toolbar. "<Empty>" means that a journal has not been assigned to that button.

Assign Entry

Selects a journal to be assigned to a journal toolbar button.

Clear Entry

Clears the assigned journal from the button currently selected in the *Journal Name* table and displays "<Empty>" for that particular button.

Clear All Entries

Clears all assigned journals from the *Journal Name* table and displays "<Empty>" for all buttons. When you you choose this button, a message window will appear, asking you to verify that you wish to clear all buttons.

Button Graphic

Opens the Button Graphic dialog box, from which you can select a graphic (a colored square or a directional arrowhead) which will appear on the currently selected button.

Wording to Appear on Button

Specifies a label for the currently selected button.

Toolbar Title

Specifies the title that appears at the top of the toolbar.

Close

Closes the Edit Journal Toolbar dialog box.

Toolbar Tab Page

Rows

Selects the number of rows in the toolbar.

Columns

Selects the number of columns in the toolbar.

Width

Selects the number of characters displayed on each toolbar button.

Toolbar Title

Specifies the title that appears at the top of the toolbar.

Close

Closes the Edit Journal Toolbar dialog box.

Edit Journal Toolbar (Journals Menu)

Assigns journals to empty toolbar buttons, replaces existing journals assigned to buttons with new journals, and clears journals from the selected toolbar. Adds new rows or columns of buttons to the selected toolbar.

Use this command when you want to add or change the journals assigned to an existing toolbar's buttons or when you want to change the size of the toolbar. This command will be unavailable until a journal toolbar has been created with the Create Journal Toolbar command.

Note: If you load an updated version of MetaFluor to a different directory from the previous version, you will need to update your old journal toolbars with the Edit Journal Toolbar command so that the toolbars will be configured to look for its journals in the appropriate directory.

See Also:

Create Journal Toolbar

Load Journal Toolbar

List of Journal Functions

Editing Journal Toolbars

To edit a journal toolbar, use the following procedure:

Step	Action
1	From the Journals menu, choose Edit Journal Toolbar. The Edit Journal Toolbar dialog box will appear. This consists of two tabbed pages, a Buttons page and a Toolbar page.
2	If you want to change the number of buttons on the Toolbar, change the configuration of rows and columns, or change the width of the buttons, select the Toolbar tab.
3	Change the number of rows or columns as desired by making an alternative selection from the *Rows* and *Columns* spin boxes, respectively.
4	To change the width of the buttons, select a new width from the *Width* spin box.
5	To change the Toolbar's title, overtype the current entry in the *Toolbar Title* text box with the new title.
6	To change the assignments of journals, graphics, or labels of the Toolbar buttons, select the Buttons tab.
7	To change the journal assignment of a button, highlight the desired button location for the journal name in the *Journal Name* list and choose *Assign Entry.*
8	The Select a Journal dialog box will appear. Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button to locate the appropriate drive and folder. Then choose *Open.*
9	When the journal name appears in the *Journal Name* table, type the text that is to appear on the button in the *Wording to Appear on Button* text box.
10	If you want to place a graphic on the button (a colored square or a directional arrowhead), or change the graphic assignment of the button, choose the *Button Graphic* command button. The Button Graphic dialog box will appear. Click the graphic of your choice, or choose *(None)* to remove an assigned graphic. The Button Graphic dialog box will close automatically. and the graphic you chose will appear on the *Button Graphic* command button for the selected entry in the *Journal Name* table.
11	Repeat Steps 7 - 10, as needed, for each button.

12   When you have finished, choose *Close.*

Edit Journal Toolbar - Dialog Box Options

Buttons Tab Page

Journal Name

Lists the names of the journals assigned to each button in the toolbar. "<Empty>" means that a journal has not been assigned to that button.

Assign Entry

Selects a journal to be assigned to a journal toolbar button.

Clear Entry

Clears the assigned journal from the button currently selected in the *Journal Name* list and displays "<Empty>" for that particular button.

Clear All Entries

Clears all assigned journals from the *Journal Name* list and displays "<Empty>" for all buttons. When you you choose this button, a message window will appear, asking you to verify that you wish to clear all buttons.

Button Graphic

Opens the Button Graphic dialog box, from which you can select a graphic (a colored square or a directional arrowhead) which will appear on the currently selected button.

Wording to Appear on Button

Specifies a label for the currently selected button.

Toolbar Title

Specifies the title that appears at the top of the toolbar.

Close

Closes the Edit Journal Toolbar dialog box.

Toolbar Tab Page

Rows

Selects the number of rows in the toolbar.

Columns

Selects the number of columns in the toolbar.

Width

Selects the number of characters displayed on each toolbar button.

Toolbar Title

Specifies the title that appears at the top of the toolbar.

Close

Closes the Edit Journal Toolbar dialog box.

Load Journal Toolbar (Journals Menu)

Loads the selected toolbar as the active toolbar.

Use this command when you want to load a different toolbar as the active toolbar. When the toolbar is loaded, you can run any of the journals assigned to its buttons.

If you were using a toolbar prior to saving the current protocol file, that toolbar will be loaded the next time the protocol file is used (this also applies to the default protocol file). This means that you may only need to use the Load Journal Toolbar command to switch between toolbars.

See Also:

Create Journal Toolbar

Loading a Journal Toolbar

To load a journal toolbar, use the following procedure:

Step	Action
1	From the Journals menu, choose Load Journal Toolbar. The Select a Journal Toolbar dialog box will appear.
2	Select the desired toolbar file. Use the *Look In* list or Up One Level button to locate the correct drive and folder, if necessary.
3	Choose *Open.* The selected toolbar will appear in the MetaFluor application window.

Load Journal Toolbar- Dialog Box Options

File Name

Lists the name of the currently selected journal file.

Files of Type

Determines the file format of the files to be displayed in the *File Name* list.

Look In

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

Open

Opens the toolbar file.

Cancel

Cancels the command.

Journal Toolbar Shortcuts (Journals Menu)

Displays a secondary menu which lists keyboard shortcuts that can be used to run the journals that are on the current toolbar.

Use this command to view the keyboard shortcuts for each journal on the toolbar. Although you can run the journals by selecting a menu item from the secondary menu, this command's purpose is to provide you with a list of the keyboard shortcuts assigned to the current journal toolbar. Shortcuts which are not assigned to a toolbar button will display the message "Not available" in dimmed text.

Once you know the shortcuts associated with each journal, you won't need to use the Journal Toolbar Shortcuts command. The keyboard shortcuts work the same way as other keyboard shortcuts listed in the menus: press and hold the first key and then press the second key listed.

EXAMPLE:

The first journal toolbar on the active toolbar might be named "Acquire." Its shortcut is CTRL + 1. Press and hold the [CTRL] key and then press the number [1] on your keyboard.

See Also:

Create Journal Toolbar

Load Journal Toolbar

Using the Journal Toolbar Shortcuts

To use the journal toolbar shortcuts, use the following procedure:

Step	Action
1	Select the Journals menu.
2	Choose Journal Toolbar Shortcuts. A secondary menu will appear. The menu lists the toolbar's journals and the keyboard shortcut assigned to each position on the toolbar. Shortcuts which are not assigned to a toolbar button will display the message "Not available" in dimmed text.
3	To run a particular journal, choose it from the secondary menu. **OR** Use the keyboard shortcut listed next to the journal: press and hold the [CTRL] key and then press the assigned numeric key.

Show Journal Toolbar (Journals Menu)

Displays the current journal toolbar.

Use this command when you want to display a toolbar that has been hidden with the Hide Journal Toolbar command. If there is no active journal toolbar, this command will be unavailable and will appear in dimmed text. Use the Load Journal Toolbar command to load a journal toolbar.

Shortcut: CTRL + J

See Also:

Hide Journal Toolbar

Load Journal Toolbar

Showing the Active Journal Toolbar

To show the active journal toolbar, use the following procedure:

Step	Action
1	Select the Journals menu.
2	Choose Show Journal Toolbar. The active toolbar will appear, and the Show Journal Toolbar command in the Journals menu will be replaced by the Hide Journal Toolbar command.
	Note: An alternative to the menu command is to use a keyboard shortcut, CTRL + J, to show or hide the toolbar. This key allows you to toggle back and forth between states.

Hide Journal Toolbar (Journals Menu)

Hides the current journal toolbar from view.

Use this command when you want to hide the current journal toolbar to gain additional desktop space or when the toolbar is not currently needed.

Shortcut: CTRL + J

See Also:

Show Journal Toolbar

Load Journal Toolbar

Hiding the Active Journal Toolbar

To hide the active journal toolbar, use the following procedure:

Step	Action
1	Select the Journals menu.
2	Choose Hide Journal Toolbar. The active toolbar will disappear, and the Hide Journal Toolbar command in the Journals menu will be replaced by the Show Journal Toolbar command.
	Note: An alternative to the menu command is to use a keyboard shortcut, CTRL + J, to show or hide the toolbar. This key allows you to toggle back and forth between states.

Windows Menu

Show or Hide Toolbar (Windows Menu)

Displays or hides the Icon Toolbar.

Use this command to display or hide the Icon Toolbar. After you have hidden the Icon Toolbar using this command, you can display it again by rechoosing the Show or Hide Toolbar command.

The Icon Toolbar provides quick access to frequently used menu commands for MetaFluor's acquisition or playback modes. You can still access these commands directly from the menu if you wish.

The Icon Toolbar is displayed in one of three modes. When you first start MetaFluor, a "start up" toolbar is shown. Click on the icons for a description of each their functions:

When you start a new experiment, the following "new experiment" Icon Toolbar will be displayed. Click on the icons for a description of each their functions:

When you open a previously stored experiment for playback, a different set of icons will be displayed in an "open experiment" Icon Toolbar. Click on the icons for a description of each their functions:
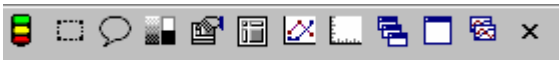
Shortcut: CTRL + T

Showing or Hiding the Icon Toolbar

To show or hide the Icon Toolbar, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Show or Hide Toolbar.
	If the Icon Toolbar is visible, it will be hidden. If it is already hidden, it will be displayed instead.

Show or Hide Command Bar (Windows Menu)

Displays or hides the Command Bar.

Use this command to display or hide the Command Bar. After you have hidden the Command Bar using this command, you can display it again by rechoosing the Show or Hide Command Bar command.

The Command Bar provides quick access to frequently used menu commands for MetaFluor's acquisition or playback modes. You can still access these commands directly from the menu if you wish. To view a description of each of the commands in the Command Bar, choose *More >>.* The Command Bar window will expand, and the *More >>* button will be replaced by the *Less <<* button. Choosing this button will condense the Command Bar again, so that it uses less space in the MetaFluor application window.

Shortcut: CTRL + B

Showing or Hiding the Command Bar

To show or hide the Command Bar, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Show or Hide Command Bar.
	If the Command Bar is visible, it will be hidden. If it is already hidden, it will be displayed instead.

Show or Hide Graphs (Windows Menu)

Displays or hides time-based graphs (measurements and analog data).

Use this command to display or hide the measurements and analog data graphs. Hiding the graphs will allow you to conserve computer memory and space on your screen without requiring you to redefine regions when you want to bring back the graph display. Graphs can be displayed again by rechoosing the Show or Hide Graphs command. The measurements graphs that will be displayed include those that have been enabled by selecting the *Show Graphs* check box in the Configure Graphs dialog box.

Shortcut: [F8]

See Also:

Configure Graphs

Showing or Hiding Graphs

To show or hide graphs, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Show or Hide Graphs.
	If the graphs are visible, they will be hidden. If they are already hidden, they will be displayed instead.

Open Sequence Status (Windows Menu)

Opens the Sequence Status window.

Use this command to display the Sequence Status window. This window displays the current experimental clock time, acquisition cycle number, and last journal run. This counter is particularly useful for keeping track of journal sequencing.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Close Sequence Status

Sequence Journals

Zero Clock

Opening the Sequence Status Window

To open the Sequence Status window, use the following procedure:

Step	Action
1	From the Windows menu, choose Open Sequence Status. The Sequence Status window will appear.
2	You can close the Sequence Status window at any time by clicking the Close button in its upper right corner, or by choosing Close Sequence Status from the Windows menu.

Sequence Status - Dialog Box Options

Clock

Displays the elapsed time from when an experiment was opened or the Zero Clock command was last carried out.

Count

Displays the current acquisition cycle.

Last Journal Run

Displays the name of the last journal to be run in the current session. If no journal has been run, this field will be blank.

Close Sequence Status (Windows Menu)

Closes the Sequence Status window.

Use this command to close the Sequence Status window. This window displays the current experimental clock time, acquisition cycle number, and last journal run.

Note: This command is unavailable in the MetaFluor Offline system.

See Also:

Open Sequence Status

Sequence Journals

Closing the Sequence Status Window

To close the Sequence Status window, use the following procedure:

Step	Action
1	From the Windows menu, choose Close Sequence Status. **OR** Clicking the Close button in the upper right corner of the Sequence Status window.
2	The Sequence Status window will close.

Open Status Window (Windows Menu)

Opens the Status window.

Use this command to display the Status window. The upper part of this window displays error messages, event marks, and Display Message journal command messages. You can clear the message at any time.

The lower half of this window displays the image saving status. The first line for the image saving status displays what will be saved if image saving is enabled (such as the entire image). The second line displays when the next image will be saved, how many more images can be saved, and the drive where the images are being saved.

If you want to display the Status window whenever you open an experiment, you can configure a preference for this in the General Preferences dialog box (Preferences command, File menu).

See Also:

Close Status Window

Preferences

Opening the Status Window

To open the Status window, use the following procedure:

Step	Action
1	From the Windows menu, choose Open Status Window. The Status window will appear.
2	Choose *Clear* whenever you want to clear a message from the Status window.

Status - Dialog Box Options

Msg

Displays error messages, event marks, and Display Message journal command messages

Save

Indicates the current image saving status (whole image, region, and so on), when the next image will be saved, how many more images can be saved, and the drive where the images are being saved.

Clear

Clears the message from the *Msg* status line.

Close

Closes the Status window.

Close Status Window (Windows Menu)

Closes the Status window.

Use this command to close the Status window. You can also close the Status window using its *Close* command button.

If you do not want to display the Status window every time you open an experiment, you can configure a preference for this in the General Preferences dialog box (Preferences command, File menu).

See Also:

Open Status Window

Preferences

Closing the Status Window

To close the Status window, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Close Status Window. The Status window will close.

Open Notebook (Windows Menu)

Opens the Notebook.

Use this command when you want to keep notes while you are working on an experiment. MetaFluor records the current date and time in the Notebook file when you open the Notebook. You can type any additional information as necessary. The Notebook is a simplified text editor: you can **copy, cut, and paste text** to and from it. **If you want to start a new line in the Notebook, hold down the [CTRL] key while pressing the [ENTER] key.** The text box can be resized as need by dragging its borders.

Note: The pop-up context menu that appears when you right-click in the Notebook window contains commands that allow you to cut, copy, paste, or delete text, as well as to undo any changes you make.

When you save images from an experiment, the corresponding Notebook file will also be saved. This is a text file (*.txt) that can be opened with the Microsoft WordPad or Notepad programs (which come bundled with Windows). In addition, the Notebook window has *Copy* and *Print* command buttons that allow you to copy the text to the Clipboard or send it to a printer. Notebook files can be saved and loaded independently using the *Save* and *Load* command buttons, respectively.

You can configure a preference in the General Preferences dialog box to open the Notebook automatically whenever an experiment is open (Preferences command, File menu).

See Also:

Preferences

Close Notebook

Opening the Notebook

To open the Notebook, use the following procedure:

Step	Action
1	Start a new experiment or open a previously stored experiment.
2	From the Windows menu, choose Open Notebook. The Notebook window will appear.
3	If you want to load a previously saved Notebook file, choose *Load* and select the icon for the desired .txt file from the Load Notebook dialog box that appears. If necessary, use the *Look In* drop-down list box or Up One Level icon button to locate the appropriate drive and folder. Then choose *Open.*
4	In the Notebook window's text box, type the text you want to save. If you alter the entry in a previously saved Notebook, the word "(Modified)" will be appended to the name in the Notebook window's title bar.
5	If you want to print the information in the Notebook window, choose *Print.* **OR** If you want to copy the information to the Clipboard so that you can paste it into another Windows-based program, choose *Copy.*
6	If you want to save the text in a Notebook file now, choose *Save.* The Save Notebook dialog box will appear. Type a name for the file in the *File Name* text box. Then choose *Save.* **OR** If you want to save the Notebook file automatically when you end the experiment, you need do nothing. The file will be saved automatically under the .inf file's name with the ".txt" extension when you end the experiment.

Open Notebook - Dialog Box Options

Text Box

Use the text box at the top to type, copy text to or copy text from the Notebook. You can

Advance One Line:

Press the [CTRL] and [ENTER] keys simultaneously with the insertion point positioned at the end of the previous line.

Select One Word:

Position the pointer above the word and double-click the left mouse button.

Cut Text:

Select the desired text and press [CTRL] + [X].

Copy Text:

Select the desired text and press [CTRL] + [C].

Paste Text:

Select the desired text and press [CTRL] + [V]

Delete Text:

Press the [BACKSPACE] or the [DEL] key.

Clear

Deletes all of the text in the Notebook.

Load

Opens the Load Notebook dialog box, from which you can load a previously saved Notebook (*.txt) file.

Save

Opens the Save Notebook dialog box, from which you can save the current Notebook (*.txt) file. **Note:** Notebook files are saved automatically when an experiment is ended. You should only need to use the *Save* command if you want to save independent Notebook entries.

Copy

Copies the information in the Notebook text box to the Clipboard, so that you can paste it into another Windows-based program.

Print

Sends the information in the Notebook text box to your default printer.

Close

Closes the Notebook.

Close Notebook (Windows Menu)

Closes the Notebook.

Use this command when you want to close the Notebook. The Notebook is used to record notes while you are working on an experiment.

When you save images from an experiment, the corresponding notebook (*.txt) file is also saved. This file is a text file and can be opened with the Windows WordPad or NotePad programs if you want to print a copy of it.

Use the **Open Notebook** command to open the Notebook again if necessary.

You can configure a preference in the General Preferences dialog box to open the Notebook automatically whenever an experiment is open (Preferences command, File menu).

See Also:

Preferences

Open Notebook

Closing the Notebook

To close the Notebook, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Close Notebook. The Notebook will close.

Open Annotation (Windows Menu)

Opens the Annotation window.

Use this command during a new experiment or during playback of a stored experiment to view a display of the annotations that are automatically made during image acquisition. The annotations will include the (1) Video acquisition, (2) Exposure time, (3) Image region size, and (4) Background subtraction and shading correction status of each image.

Note: The pop-up context menu that appears when you right-click in the Annotation window contains commands that allow you to cut, copy, paste, or delete text, as well as to undo any changes you make.

See Also:

Close Annotation

Opening the Annotation Window

To open the Annotation window, use the following procedure:

Step	Action
1	Start a new experiment or open a previously stored experiment.
2	From the Windows menu, choose Open Annotation. The menu item will change to Close Annotation, and the Annotation window will appear. The annotations for the currently open images will be displayed in the main text box in the lower portion of the dialog box.
3	To view a display of a particular image, select the image window from the *Image* drop-down list box.
4	To view the annotations for images at a different timepoint in an experiment being played back, use the **Experiment Control Panel** to switch to the desired timepoint.

Open Annotation - Dialog Box Options

Image

Selects the image whose annotation you want to display.

Annotation text box

Displays information regarding the selected image's video acquisition mode, exposure time, size, and background subtraction and shading correction status.

Close Annotation (Windows Menu)

Closes the Annotation window.

Use this command when you want to close the Annotation window. The Annotation window is used to view a display of annotations that are made automatically during image acquisition. The annotations will include the (1) Video acquisition, (2) Exposure time, (3) Image region size, and (4) Background subtraction and shading correction status of each image.

See Also:

Open Annotation

Closing the Annotation Window

To close the Annotation window, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Close Annotation. The Annotation window will close.

Arrange Windows (Windows Menu)

Resizes and tiles all open image windows so that they fit across the top of the MetaFluor application workspace.

Use this command when you want to optimize space on the desktop. The image windows will be resized to fit in a single row across the application window.

See Also:

Bring Images to Front

Arranging the Image Windows

To arrange the image windows to fit in a single row, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Arrange Windows. All open image windows will be tiled horizontally across the upper edge of the MetaFluor application workspace.

Bring Images to Front (Windows Menu)

Brings image windows to the uppermost layer of the workspace display.

Use this command when your image windows are obscured by dialog boxes or graph windows and you need to see the entire images without obstruction. This command is particularly handy when your workspace is cluttered with dialog boxes or graph windows that, because of their size or numbers, obscure your view of the image windows.

If you have overlapping images in your workspace, successive uses of this command will bring images to front by alternating between the "lowest" and "highest" windows in the pile.

Note: Some dialog boxes are "modal"; that is, they must be closed before you can use another command. There will therefore be some conditions under which you will not be able to take advantage of the "Bring to Front" commands.

Shortcut: CTRL + I

See Also:

Bring Dialogs to Front

Bring Graphs to Front

Bringing Images to Front

To bring an otherwise obscured image window to the "top" of your workspace display, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Bring Images to Front. The obscured image window will be displayed in the topmost layer of the workspace display.
3	As an alternative, you can use the keyboard shortcut, CTRL + I.

Bring Dialogs to Front (Windows Menu)

Brings dialog boxes to the uppermost layer of the workspace display.

Use this command when your workspace is cluttered with image or graph windows that, because of their size or numbers, obscure your view of the command dialog box. This command is most effectively used for bringing the Experiment Control Panel to the front.

Note: Some dialog boxes are "modal"; that is, they must be closed before you can use another command. There will therefore be some conditions under which you will not be able to take advantage of the "Bring to Front" commands.

Shortcut: CTRL + D

See Also:

Bring Images to Front

Bring Graphs to Front

Bringing Dialogs to Front

To bring an otherwise obscured dialog box to the "top" of your workspace display, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Bring Dialogs to Front. The obscured dialog box will be displayed in the topmost layer of the workspace display. **Note:** This command is most effectively used for bringing the Experiment Control Panel to the front.
3	As an alternative, you can use the keyboard shortcut, CTRL + D.

Bring Graphs to Front (Windows Menu)

Brings the graph windows to the uppermost layer of the workspace display.

Use this command when your workspace is cluttered with image windows and dialog boxes that, because of their size or numbers, obscure your view of the graph windows.

Note: Some dialog boxes are "modal"; that is, they must be closed before you can use another command. There will therefore be some conditions under which you will not be able to take advantage of the "Bring to Front" commands.

Shortcut: CTRL + G

See Also:

Bring Images to Front

Bring Dialogs to Front

Bringing Graphs to Front

To bring your otherwise obscured graph windows to the "top" of your workspace display, use the following procedure:

Step	Action
1	Select the Windows menu.
2	Choose Bring Graphs to Front. The obscured graph windows will be displayed in the topmost layer of the workspace display.
3	As an alternative, you can use the keyboard shortcut, CTRL+ G.

Analog Menu

Introduction to the Analog Measurements Drop-in

Overview

The Analog Measurements drop-in, DAC, supplies an additional menu of MetaFluor commands for acquisition of voltage data in addition to, or in lieu of, acquisition of images. Sources of the voltage data can be simple measurement probes, such as a calcium or oxygen electrode, or they can be multiplexed devices for continuous measurement, such as are used in electrophysiology. MetaFluor can acquire the voltage data either at a particular time during the acquisition cycle, or continuously at a user-specified rate during the experiment.

Graphing, Logging, and Saving Analog Data

Analog data acquired by MetaFluor can be graphed, logged, and/or saved to a file. Each measurement channel, from as many as 16 channels, can have its own data column in a log file and its own scrolling, time-based graph. Any analog data that have been saved to a .bin file can be replayed for offline analysis. In addition, two channels can be "ratioed" and the ratio represented on another configurable, time-based graph. (However, the numeric format of ratio data prevents the saving of analog ratio values in a .bin file.)

Equipment Required for Analog Measurements

To perform analog measurements in MetaFluor, you will need the Analog Measurements drop-in, DAC.out, a National Instruments data acquisition (DAQ) board such as the PC-LPM-16 or LAB-PC+ boards, and your own measurement probes or other hardware which generate analog signals suitable for use with the data acquisition board.

Prior to Using the Analog Measurements Drop-in

First you will need to install the National Instruments data acquisition board and the National Instruments software drivers. (Follow the instructions provided in the National Instruments documentation that was included with the board.)

Then you will need to install the Analog Measurements drop-in using the **MetaMorph Meta Imaging Series Administrator.** The name for this drop-in is *DAC.* After you have installed the drop-in, you can start MetaFluor.

Finally, you will need to install the hardware driver for the drop-in and configure a Data Acquisition Device to be able to make analog measurements. You can do this from the dialog box that opens when you choose **Configure Analog Measurements** from the Analog menu. Alternatively, you can use the Illumination Control command from the Devices menu to **install and configure** the hardware driver.

Configure Analog Measurements (Analog Menu)

Configures the device(s) supplying analog data to the computer.

Drop-in: DAC

Use this command to configure the hardware used for acquiring analog measurement data and to configure the display and storage of analog measurement data. This function allows MetaFluor to act like an analog-to-digital converter (ADC), acquiring data from such source signals as pH meters, calcium or oxygen electrodes, temperature probes, and electrophysiological amplifiers. Data can be acquired at a user-specified stage in the ratiometric acquisition cycle, or it can be acquired asynchronously at a user-specified rate.

Note: You must first **start a new experiment** for this command to be enabled.

When you configure your data acquisition, you will select the data channel to be associated with each "virtual" channel that is acquired by MetaFluor. Alternatively, you can select a ratio of two data channels, or create your own equation that uses any combination of data channels and arithmetic operators, constant values, logical operators, or Boolean functions. This flexibility allows you, for example, to perform shading correction and background subtraction on each of two Wavelength inputs and then plot the ratio of the two sets corrected images.

QUICK TIP: You can hide and redisplay the analog data graphs by choosing Show or Hide Graphs from the Windows menu or by using the keyboard shortcut, the [F8] function key.

See Also:

Overview of the Use of Configure Analog Measurements

Analog Display

Analog Async Status

Replay Stored Data

Convert Data File to Text File

Overview of the Use of the Configure Analog Measurements Command

To use Configure Analog Measurements, use the following procedure:

Step	Action
1	From the Analog menu, choose Configure Analog Measurements. The Configure Analog Measurements dialog box will appear.
2	If you have not already done so, you must install the appropriate hardware driver. Exit Metafluor and open the Meta Imaging Series Administrator and follow the instructions in the help file for yor specific device.
3	Also if you have not done so, you must install and configure a Data Acquisition Device.
4	In the Configure Analog Measurements dialog box, define your virtual measurement channels. Follow the procedure for **defining virtual analog measurements channels.**
5	After defining a channel, you must decide if its data are to be displayed in a real-time graph, saved as text to a log file, or saved as binary data to a Save file (*.bin). If you want to display the data from the channel in an on-screen graph, follow the procedure for **configuring the analog data graph.** If you want to save the data from the channel as text to a log file, follow the procedure for **configuring the analog measurements log.** If you want to save the data from the channel as binary data to a Save file, follow the procedure for **configuring an analog measurements Save file.**
6	Next you will need to calibrate the measurement channel. Follow the procedure for **calibrating analog measurements channels.**
7	Repeat Steps 5 - 7 for each measurement channel.
8	When you have finished, choose *Close.* You will now be able to acquire analog data automatically when you run experiments in MetaFluor.

Defining Virtual Analog Measurements Channels

You will need to define a virtual measurement channel for each analog measurement you want to make, up to a maximum of 16. You can associate any number of virtual channels with the same physical channel.

To define a virtual channel for measurement of analog data, use the following procedure:

Step	Action
1	In the Configure Analog Measurements dialog box, locate the Virtual Channel List Box.
2	Double-click the virtual channel number you want to use. If a channel is already in use, a check mark will be seen next to its entry.
3	You next need to associate the virtual channel with a physical channel. Select one of the physical channels listed in the *Measure* drop-down list. If additional configuration is required, the *Configure* button will become available.
	If you select *Ratio of 2 Channels,* you can assign a ratio of any two data channels to the virtual channel you are configuring. The *Calibrate Channel* button will become a *Configure Ratio* button, and the *Save Data* check box will become unavailable.
	If you select *Formula,* you can create an equation using any of the data channels and arithmetic operators (+, -, *, /), constant values, logical operators (<, >, =, <>, >+, <+), or Boolean functions (AND, OR, ABS, IF). The *Calibrate Channel* button will become an *Edit Formula* button, and the *Save Data* check box will become unavailable.
4	Choose *Configure* and enter the specifications for your hardware device in the dialog box that appears. Refer to the device's owner's manual for the required configuration parameters.
5	From the *Sample* drop-down list, select the time during the experiment when the channel is to acquire data.
6	If you selected *Asynchronously* in Step 5, a new control spin box, *Sampling Frequency,* will appear. Select the desired rate of acquisition from this spin box.
7	If you selected *Ratio of 2 Channels* in Step 3, choose the *Configure Ratio* button. The Configure Ratio dialog box will appear.
	AND Select the appropriate channels from the numerator (upper) and denominator (lower) drop-down lists. Choose *OK* to return to the Configure Analog Measurements dialog box

when you have finished.

8   If you selected Formula in Step3, choose the
     Edit Formula button. The Edit Formula dialog
     box will appear.

     AND
     Type a formula in the text box in the upper part
     of the dialog box. You can use any
     combination of data channels, arithmetic
     operators, logical operators, Boolean
     functions, and constants in your equation. To
     test its validity, choose the *Evaluate Formula*
     button. Choose *OK* to return to the Configure
     Analog Measurements dialog box when you
     have finished.

Configuring the Analog Data Graphs

To configure a graph for real-time, on-screen display of the data from an analog measurements channel, use the following procedure. The X-axis will always represent time.

Note: You can hide and redisplay the analog data graphs by choosing Show or Hide Graphs from the Windows menu or by using the keyboard shortcut, the [F8] function key.

Step	Action
1	In the Configure Analog Measurements dialog box, select the *Graph Data* check box, so that a check mark appears in it.
2	Choose *Configure Graph.* The Configure Graph dialog box will appear.
3	Type a title for the graph in the *Graph Title* text box.
4	Type a label for the Y-axis in *Title* text box.
5	Type a minimum and maximum value for the range of the Y-axis in the *Minimum Value* and *Maximum Value* text boxes, respectively.
6	To specify the number of major intervals in the Y-axis, enter a value in *Major Tick Marks.* **AND** To specify the number of minor tick marks to be displayed between each major tick mark on the Y-axis, enter a value in *Minor Tick Marks.*
7	Use the *Maximum Points on the Graph* spin box to specify the number of data points to appear at a time on the updating analog measurements graph.
8	If you do not want to graph every data point, you can use data reduction to specify that only every nth data point be graphed. To do so, select the "skip" factor from the *Reduce Points by a Factor of* spin box. **AND** From the *When Reducing Data* option button group, select a value for each data-reduced point on the graph: the lowest value *(Use Mimimum Point in Data Set),* the highest value *(Use Maximum Point in Data Set),* or an average *(Use Average Value of Data Set).*
9	When you have finished, choose *OK.*

Configure Graph - Dialog Box Options

Graph Title

Specifies a title for an analog data graph.

Title

Specifies a label for the Y-axis.

Minimum Value

Defines the minimum value for the Y-axis range.

Maximum Value

Defines the maximum value for the Y-axis range.

Major Tick Marks

Specifies the number of major intervals in the Y-axis.

Minor Tick Marks

Specifies the number of minor tick marks to be displayed between each major tick mark on the Y-axis.

Maximum Points on the Graph

Specifies the number of data points to appear at a time on the updating analog measurements graph.

Reduce Points by a Factor of

Selects a "skip" factor to specify that only every nth data point is graphed.

When Reducing Data

Selects a value for each data-reduced point on the graph: the lowest value *(Use Mimimum Point in Data Set),* the highest value *(Use Maximum Point in Data Set),* or an average *(Use Average Value of Data Set).*

OK

Accepts the graph settings and closes the Configure Graph dialog box.

Cancel

Rejects the graph settings and closes the Configure Graph dialog box.

Configuring the Analog Measurements Log

To configure the analog measurements log, use the following procedure. (The log file itself is defined and opened automatically when you open the Experiment Control Panel from the Run Experiment menu and select its *Log Data* check box.)

Note: Asynchronously acquired analog measurements data can not be logged. Data can be logged only if sampling is conducted at a specific time during the acquisition cycle. Asynchronously acquired data should be saved to a Save file.

Step	Action
1	In the Configure Analog Measurements dialog box, select the *Log Data* check box so that a check mark appears in it.
2	Choose *Configure Log.* The Configure Log dialog box will appear.
3	Type a title in the *Column Title* text box for the column in the data file or worksheet to which data will be logged.
4	Type a description of the data in the *Description* text box. The text you enter will be displayed next to the column title you specified in Step 3.
5	In the *Data Format* text box, specify the number of digits to be output to the data log.
6	When you have finished, choose *OK.*

Configuring the Analog Measurements Save File

To save analog measurements to a binary Save file (*.bin), use the following procedure. This file can be replayed later to analyze a stored experiment (see **Replay Stored Data).** Note: Because of the numeric format used for representing data, ratio values obtained from "ratioing" a pair of analog channels can not be saved in a .bin file, and the *Save Data* check box will be unavailable.

Step	Action
1	In the Configure Analog Measurements dialog box, select the *Save Data* check box so that a check mark appears in it.
2	Choose *Configure Save.* The Configure Save dialog box will appear.
3	Choose *Select Save File.* The Select Save File dialog box will appear.
4	You can either choose an existing .bin data file by selecting its icon, or you can type a name in the *File Name* text box for a new file to be created. If necessary, use the *Save In* drop-down list or Up One Level icon button to change to the correct location. Then select the icon for the file and choose *Save.*
	The *File Status* text field will indicate if a file already exists and that data will be appended to it, or it will indicate that the file does not exist and will be created.
5	If you wish, you can rename a file by choosing *Rename Existing File.* The Rename dialog box will open.
	AND
	Type the new file name in the *New Filename* text box and choose *OK.*
6	If you want to delete a file that already exists, choose *Delete Existing File.* A dialog box will appear, asking you to verify the deletion. Choose *Yes* to confirm deletion.
7	When you have finished, chose *Close.*

Calibrating the Analog Measurements Channels

To calibrate an analog measurements channel, use the following procedure:

Step	Action
1	From the Configure Analog Measurements dialog box, choose *Calibrate Channel.* The Calibrate Channel dialog box will appear.
2	You must have at least two "known" samples to perform a calibration, and you can have as many as eight "knowns." Select a check box in the leftmost column for each "known" sample. This will enable the corresponding *Sample* buttons.
3	Next, for each "known" (standard) sample, put your measurement probe into the sample and press the *Sample* button for that sample. The measured value, in raw units, will appear in the *Measured* text box next to the *Sample* button.
4	Now type the known value in the *Known* text box.
5	Repeat Steps 3 and 4 for each "known" sample.
6	Select the type of curve-fitting you want from the *Curve Fit* drop-down list.
7	If needed, select the *Extrapolate, Enforce Maximum Limit,* or *Enforce Minimum Limit* check boxes and enter values in the associated spin boxes to extrapolate the curve or to limit it to a specific range.
8	Choose *Auto-Calculate Graph Axes* to create the calibration curve.
9	If you change any of the *Measured* or *Known* fields and do not want to change the graph axes, choose *Update Graph* to recalculate and redraw the graph.
10	When you have finished, choose *Close.*

Calibrate Channel - Dialog Box Options

Use

Selecting these check boxes will in turn enable the corresponding *Sample* buttons. One of these should be selected for each "known" sample used in the calibration. At least two "knowns" are needed to perform a calibration, and you can use as many as eight.

Sample

These "buttons" are chosen after loading a standard sample. The sample will be measured, and the measured value, in raw units, will appear in the corresponding *Measured* text box.

Measured

After you press the corresponding *Sample* button, each of these text boxes will automatically display the measured value, in raw units, of the standard sample that has been loaded.

Known

The known value of the standard sample, in actual units, is typed in this text box.

Curve Fit

This drop-down box allows the selection of the type of curve-fitting to be performed in the calibration procedure. Choices are: *Linear Interpolation, Polynomial Interpolation, Line of Best Fit, Line of Best Log, 3rd Degree Polynomial,* and *4th Degree Polynomial.*

Extrapolate

Selecting this check box allows extrapolation of the calibration curve beyond the data points. Be sure to define the desired range with *Enforce Maximum Limit* and *Enforce Minimum Limit.*

Enforce Maximum Limit

Selecting this check box and entering a value in the spin box allows the specification of the maximum value of the range of the calibration curve.

Enforce Minimum Limit

Selecting this check box and entering a value in the spin box allows the specification of the minimum value of the range of the calibration curve.

Linear Interpolation Graph

Displays the calibration curve in graphical format.

Update Graph

If any of the *Measured* or *Known* fields have been changed, but you do not want to change the graph axes, choosing this option will recalculate and redraw the graph.

Auto-Calculate Graph Axes

Creates the calibration curve from the *Measured* and *Known* data values.

Close

Closes the Calibrate Channel dialog box.

Configure Analog Measurements - Dialog Box Options

Virtual Channel List

Lists the available virtual channels (maximum of 16) for measurement of analog data. You can associate more than one virtual channel with an individual physical device channel. For example, you can have a single pH measurement probe attached to a specimen, but may want to record the pH separately for each excitation wavelength. The pH data for each wavelength can thus be displayed in its own graph. Each channel can be enabled or disabled with a double-click. When a particular virtual channel is selected, all option settings in the rest of the dialog box will pertain to that virtual channel.

Measure

Selects a physical hardware channel to be associated with a virtual channel. Alternatively, you can select *Ratio of 2 Channels* to take a "ratio" between two analog data channels, and graph the ratio values on another analog measurements graph, or you can select *Formula* to create an expression using any combination of data channels, constant values, arithmetic operators, logical operators, and Boolean functions. If you select *Ratio of 2 Channels,* the *Calibrate Channel* button will become the *Configure Ratio* button. If you select *Formula,* the *Calibrate Channel* button will become the *Edit Formula* button.

Sample

Specifies a time during the experimental acquisition cycle when a virtual channel is to receive analog data. You can acquire before or after the entire acquisition cycle. Alternatively, you can acquire before, during, or after the acquisition period for Wavelength 1, 2, or 3. Finally, you can acquire asynchronously (not associated with a particular time in the acquisition cycle) throughout the experiment. If you choose *Asynchronously,* an additional option, *Sampling Frequency,* will appear, allowing you to specify the rate of acquisition in Hz. Asynchronous sampling can accommodate continuous rates of up to 10 kHz, with a maximum rate of up to 50 kHz.
Note: If you select *Asynchronously,* you will not be able to send the data to a log file. Instead, you should save your data to a binary Save file.

Graph Data

Selecting this check box allows data from the currently selected virtual channel to be displayed in a real-time, on-screen graph.

Configure Graph

Opens the Configure Graph dialog box, from which you can specify graph titles, labels, ranges, and the number of tick marks.

Log Data

Selecting this check box allows the data from the currently selected virtual channel to be logged to a text-based log file or by Dynamic Data Exchange to an open spreadsheet.

Configure Log

Opens the Configure Log dialog box, allowing you to specify a title and numeric format for the analog data column in the log file.

Save Data

Selecting this check box allows the data from the currently selected virtual channel to be saved to a binary (*.bin) Save file. Data saved in this format can be replayed at a later time for offline analysis. This option will be unavailable for virtual channels configured for representation of a ratio between two analog input channels.

Configure Save

Opens the Configure Save dialog box, allowing you to select, rename, or delete a Save file.

Calibrate Channel

Opens the Calibrate Channel dialog box, allowing you to calibrate the analog signal of a virtual channel. Even if a previous virtual channel has been associated with particular physical channel (selected in the *Measure* drop-down list) and the calibration procedure has been performed, selection of a different virtual channel for that same physical channel will still require that the calibration procedure be performed once again.

Configure Ratio

If you select *Ratio of 2 Channels* from the *Measure* drop-down list, the *Calibrate Channel* button will become the *Configure Ratio* button. This button opens the Configure Ratio dialog box, from which you can select a pair of channels, one representing the "numerator" and the other the "denominator."

Edit Formula

If you select *Formula* from the *Measure* drop-down list, the *Calibrate Channel* button will become the *Edit Formula* button. This button opens the Edit Formula dialog box, from which you can create an equation involving any combination of data channels, arithmetic operators, constant values, logical operators, or Boolean functions. The value of the formula will be calculated and plotted on the analog measurements graph just as if it were data from a physical data channel. This flexibility allows you, for example, to perform shading correction and background subtraction on each of two Wavelength inputs and then plot the ratio of the two sets corrected images.

Close

Closes the dialog box.

Configure Log - Dialog Box Options

Column Title

Specifies a title for the analog data column.

Description

Allows a brief description of the analog data, to be displayed next to the *Column Title.*

Data Format

Allows the configuration of the digits displayed in the analog data column. Each "#" symbol corresponds to a digit. For example, the format "##.##" will cause the number 6.437 to be displayed as 6.44. Leading 0's are not printed.

OK

Accepts the data log configuration and closes the Configure Log dialog box.

Cancel

Rejects the data log configuration and closes the Configure Log dialog box.

Configure Save - Dialog Box Options

Select Save File

Opens the Select Save File dialog box, allowing you to enter a name for a new Save file or to select an existing file to which data will be appended.

File Status

This status line indicates whether a file already exists and that data will be appended to it, or if the file does not exist and will be created.

Rename Existing File

Opens the Rename dialog box, allowing you to rename an existing Save file by typing a new name in the *New Filename* text box.

Delete Existing File

Allows you to delete an existing Save file. If you choose *Delete Existing File,* a dialog box will appear, asking for confirmation.

Close

Closes the Configure Save dialog box.

Configure Ratio - Dialog Box Options

Ratio =

The upper and lower drop-down lists select data channels for the numerator and denominator, respectively, to be ratioed and plotted in the analog measurements graph.

OK

Accepts the configured ratio and closes the dialog box.

Cancel

Ignores the configuration and closes the dialog box.

Edit Formula - Dialog Box Options

Enter a Formula… (text box)

This box is used for creating an expression using any combination of data channels, constant values, arithmetic or logical operators, or Boolean functions. The equation will be calculated and plotted in the analog measurements graph, just as if it represented "raw" data being acquired by a physical data channel.

Evaluate Formula

Checks the entered formula for validity. The status line next to this button provides feedback from the evaluation.

OK

Accepts the configured formula and closes the dialog box.

Cancel

Rejects the formula and closes the dialog box.

Analog Display (Analog Menu)

Configures the display of analog data graphs.

Drop-in: DAC

Each virtual analog data channel has its own associated graph. This can result in up to 16 graphs appearing simultaneously on your screen. This command allows you to show or hide analog data graphs during an experiment. You can also use this command to clear a graph or to reconfigure its X or Y axis. Configuring the X-axis will affect all displayed virtual channel graphs.

QUICK TIP: You can hide and redisplay the analog measurements graphs by choosing Show or Hide Graphs from the Windows menu or by using the keyboard shortcut, the [F8] function key.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Configure Graphs

Configuring the Analog Display

To configure the display of your analog data graphs, use the following procedure.

Note: You can also hide and redisplay the analog data graphs by choosing Show or Hide Graphs from the Windows menu or by using the keyboard shortcut, the [F8] function key.

Step	Action
1	From the Analog menu, choose Analog Display. The Analog Display dialog box will appear.
2	From the *Analog Channel* drop-down list, select the virtual channel associated with the graph you want to configure.
3	If you want to hide the graph from view during the experiment or during playback, choose *Hide Graph.* **OR** If you decide to display a graph after hiding it, you can choose *Show Graph.* This action will not affect the data being acquired and stored.
4	If you want to erase all the data points on a graph, choose *Clear Graph.*
5	If you want to reconfigure the Y-axis of a graph, choose *Config Y Axis.* The Configure Graph dialog box will appear. Enter any changes you want to make to the graph title or Y-axis label, range, or tick marks. Choose *OK* when you have finished.
6	Repeat Steps 2 - 5 for each channel.
7	If you want to reconfigure the X-axis, choose *Config X Axis.* The **Configure Graphs dialog box** will open. Enter the desired X-axis units and range in the *Time* and *Range* boxes, respectively, and choose *OK.*
8	When you have finished, choose *Close.*

Analog Display - Dialog Box Options

Analog Channel

Selects the channel to be associated with an analog data graph.

Show Graph

Enables the display of the selected graph.

Hide Graph

Disables the display of the selected graph. This will not affect the data being acquired and stored.

Clear Graph

Erases all the data points plotted on a graph.

Config Y Axis

Opens the Configure Graph dialog box, allowing you to change the title of the graph or the Y-axis label, range, or tick marks. This is the same dialog box as is seen when *Configure Graph* is chosen from the Configure Analog Measurements dialog box.

Config X Axis

Opens the Configure Graphs dialog box, allowing you to change the X-axis units and range. This is the same dialog box as appears when you choose **Configure Graphs** from the Graphs menu. By default, Graph 2 will be the active graph selected for configuration from the *Graph* drop-down list. Any changes you make will affect all displayed virtual channel graphs.

Close

Closes the Analog Display dialog box.

Analog Async Status (Analog Menu)

Toggles the active/inactive state of an asynchronously sampling analog data channel.

Drop-in: DAC

Use this command to control whether a virtual analog data channel, running in asynchronous mode, is actively acquiring data during an experiment. By default, asynchronously sampling virtual analog channels will measure data as long as a MetaFluor experiment is running. The dialog box that appears when you choose Analog Async Status will allow you to turn such sampling on or off.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Defining Virtual Analog Measurements Channels

Controlling Analog Async Status

To use the Analog Async Status control dialog box, use the following procedure:

Step	Action
1	From the Analog menu, choose Analog Async Status. The Analog Async Status dialog box will appear.
2	Sixteen check boxes will be seen, corresponding to the 16 virtual channels. While a check box is selected, that channel will actively measure data.
	To stop a channel from gathering data, clear its check box with a mouse click.
	To switch an inactivated channel back on, reselect its check box with another click.
	(Channels which are not acquiring in asynchronous mode will have their check boxes dimmed.)
3	When you have finished, choose *Close.*

Analog Async Status - Dialog Box Options

Virtual Channel Check Boxes

Selecting a channel's check box permits that channel to acquire data, while clearing its check box stops that channel from acquiring data. Channels that are not running in asynchronous mode will have their check boxes dimmed.

Close

Closes the dialog box.

Replay Stored Data (Analog Menu)

Allows you to replay analog data which was stored in binary format in a Save file (*.bin).

Drop-in: DAC

Use this command to select a previously saved experiment in a Save file for offline playback of analog data.

Note: You must first **open the experiment** by selecting its associated Information file (*.inf).

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Configuring the Analog Measurements Save File

Replaying Stored Analog Measurement Data

To replay stored analog measurement data, use the following procedure:

Step	Action
1	From the Analog menu, choose Replay Stored Data. The Replay Data dialog box will appear.
2	Choose *Select Data File.* The Select File dialog box will open.
3	Select the icon for the Save file (*.bin) that you want to open. If the file is not displayed, use the *Look In* drop-down list box or Up One Level button to locate the correct drive and folder. Then choose *Open.* The Select File dialog box will close. When you have selected a data file, all of the experiments that have been stored in it will be displayed in the Experiments List. If Analog Measurements data have been stored in an experiment, this will be indicated in the second column by the acronym DAC, and the virtual channels that were active will be listed.
4	Select an experiment from the Experiments List.
5	Choose *OK.* The Replay Data dialog box will close and the analog data will be replotted on the analog measurements graphs.

Replay Stored Data - Dialog Box Options

Select Data File

Opens the Select File dialog box, allowing you to select a Save file (*.bin) for playback of one of its experiments.

Experiments in Data File

This column in the Experiments List displays the date and time of each experiment that was saved in the Save file. These are obtained from the timestamp at the time the New Experiment command was invoked for the experiment.

Contents

This column in the Experiments List displays the data that have been stored in the experiment. If analog measurements data were stored in the experiment, this will be indicated by the acronym DAC, and the virtual channels that were active will be listed.

OK

Accepts the selected experiment for playback.

Cancel

Cancels the command.

Convert Data File to Text File (Analog Menu)

Converts experimental data from binary format to text format.

Drop-in: DAC

Use this command to convert experimental data from a Save file (*.bin) into readable, comma-delimited text, which is then saved to a text file (*.txt). This conversion could take several minutes if the binary file is large.

See Also:

Installing Drop-ins Using the Meta Imaging Series Administrator

Converting Analog Measurement Data Files to Text Files

To convert analog measurement data files to text files, use the following procedure:

Step	Action
1	From the Analog menu, choose Convert Data File to Text File. The Convert Data File to Text File dialog box will appear.
2	Choose *Select Data File.* The Select File dialog box will appear.
3	Select the icon for the Save file (*.bin) that you want to convert to text format. If the file is not displayed, use the *Look In* drop-down list box or Up One Level icon button to locate the correct drive and folder. Then choose *Open.* The Select File dialog box will close.
4	Select an experiment from the Experiment List Box.
5	Choose *Select Text File.* The Select File dialog box will open.
6	In the *File Name* list, type the name of the text file to be used to store the data. Then choose *Save.* The Select File dialog box will close.
7	Choose *OK.* The Convert Data File to Text File dialog box will close and the binary format data from the Save file will be converted to text format.

Convert Data File to Text File - Dialog Box Options

Select Data File

Opens the Select File dialog box, allowing you to select a Save file (*.bin) for conversion to text format.

Experiments in Data File

This column in the Experiments List displays the date and time of each experiment that was saved in the Save file. These are obtained from the timestamp at the time the New Experiment command was invoked for the experiment.

Contents

This column in the Experiments List displays the data that have been stored in the experiment. If analog measurements data were stored in the experiment, this will be indicated by the acronym DAC, and the virtual channels that were active will be listed.

Select Text File

Opens the Select File dialog box, allowing you to select a text file (*.txt) for conversion of experimental data from binary format.

OK

Accepts the selected experiment for conversion to text format.

Cancel

Cancels the command.

Help Menu

Updates History (Help Menu)

Displays a history of all of the updates that have been downloaded to your imaging system.

Use this command to read a list of the updates and program "patches" that have been downloaded to the imaging program. The Updates History dialog box displays a table listing the update or patch ID code, the date it was created, and the date it was installed on the computer.

EXAMPLE: "T10148,01/01/98,03/05/98" indicates that a patch with the code "T10148" and having a creation date of January 1, 1998, was installed on March 5, 1998.

Updates History - Dialog Box Options

History Table

Displays a list of all updates and patches that have been installed.

OK

Closes the dialog box.

Reading Your Updates History

To read a list of the updates and patches that you have downloaded to your imaging system, use the following procedure:

Step	Action
1	From the Help menu, choose Updates History. The Updates History dialog box will appear.
2	The dialog box displays a table that lists all updates and patches that have been installed.
	EXAMPLE: "T10148,01/01/98,03/05/98" indicates that a patch with the code "T10148" and having a creation date of January 1, 1998, was installed on March 5, 1998.
3	When you have finished reading the list, choose *OK* to close the dialog box.

Basic Tools

Dialog Boxes

Dialog boxes are displayed in MetaFluor whenever it is necessary to request information about a command or task to be performed or to supply you with information.

Commands which display a dialog box are marked with an ellipsis (". . .") in their respective MetaFluor menus. An ellipsis is also used on command buttons in dialog boxes that open secondary dialog boxes.

Most MetaFluor dialog boxes use options and command buttons that are similar to those used in many Windows-based applications. An option can be selected by positioning the pointer over it and clicking the left mouse button. If you want to use the keyboard, you can press the [Tab] key to move forward (left to right, top to bottom) to the next area within the dialog box. The cursor (Arrow) keys can be used to move between options in a group box.

Typical Dialog Box Options

Dialog boxes often have an *OK* button, a *Close* button, or another command button that closes the dialog box while completing the command. Some, however, use the Close button found in the dialog box's upper right corner. Clicking the Close button automatically closes the dialog box. To cancel a command, choose *Cancel* from the dialog box or press the [Esc] key.

TIP: To bring otherwise obscured dialog boxes to the "top" of your workspace display, use the Bring Dialogs to Front command (Windows menu), or use its keyboard shortcut, [CTRL] + [D]. This command is best used to bring the Experiment Control Panel to the front.

Selecting Files from Standard Dialog Boxes That Open and Save Files

Many MetaFluor dialog boxes that open and save files share a design common to many Windows-based programs. Once you have learned how to use these options for one dialog box, such as the one used by the Open command, you will have learned how use the options for many dialog boxes.

Selecting the Drive

Selecting the File's Directory

Selecting the File Type

Selecting the File

Icon Toolbar

This ribbon of command icons offers a quick and convenient way to carry out frequently used commands for MetaFluor's acquisition or playback modes. You can still access these commands directly from the menu if you wish.

The Icon Toolbar is displayed in one of three modes. When you first start MetaFluor, a "start up" toolbar is shown. Click on the icons for a description of each their functions:

When you start a new experiment, the following Icon Toolbar will be displayed. Click on the icons for a description of each their functions:

When you open a previously stored experiment for playback, a different set of icons will be displayed. Click on the icons for a description of each their functions:
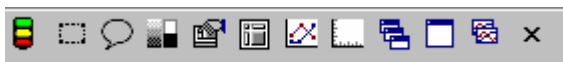
Shortcut: CTRL + T

Using Image Window Tools

Image Window Toolbar

Each image window has its own Image Window Toolbar, located on the left side of the image window, which consists of tools that operate only within that particular image window. All of the tools listed below are available for 8-bit and 16-bit images. The Zoom and Histogram Tools are the only tools available for working with a binary (1-bit) image.

Note: You can hide the Image Window Toolbar by right-clicking in the image window and choosing Hide Image Window Toolbar from the pop-up context menu that appears. The command will change to Show Image Window Toolbar. To display the toolbar once again, simply right-click again and choose Show Image Window Toolbar.

Zoom Tool

Histogram Tool

Display Mode Tool

Contrast Tool and Slider

Palette Tool

Threshold Tool and Slider

Zoom Tool (Image Window Toolbar)

The Zoom Tool provides eight levels of magnification ranging from 25 to 800 percent.

Use this tool to increase or decrease the magnification for the current image. You can select 25, 33, 50, 75, 100, 150, 200, 400, or 800 percent magnification. If you increase the magnification to 150 percent or more, you can use the Zoom Tool's "magnifying glass" pointer to select and directly zoom into a specific area of interest.

You can also use the keyboard shortcuts to increase or decrease the magnification level of the active image window. Press the appropriate shortcut key listed below until you reach the desired magnification level.

Shortcuts:

Increase magnification = [PgUp]

Decrease magnification = [PgDn]

Using the Zoom Tool

To use the Zoom Tool to increase or decrease the magnification in an image, use the following procedure:

Step	Action
1	Select the Zoom Tool by clicking it using the left mouse button.
2	Choose one of the following magnification levels from the pop-up menu that appears: 25%, 33%, 50%, 75%, 100%, 150%, 200%, 400%, or 800%. (A check mark indicates the currently chosen zoom level.)
3	If you chose 150%, 200%, 400%, or 800%, the pointer will change to a magnifying glass. Position the magnifying glass over the center of the area you want to magnify and click.
4	MetaFluor will increase or decrease the magnification.

Histogram Tool (Image Window Toolbar)

The Histogram Tool displays a distribution of an image's grayscale values.

Use this tool when you want to see a visual representation of the distribution of an image's grayscale values. A typical histogram is shown below.

In addition to creating histograms of 8-bit images, you can create histograms of 16-bit images.

Note: If you set the grayscale histogram scaling and axis range for an image with its Histogram Tool, this will update the settings for the selected wavelength in the Scale 16-Bit Images dialog box (if autoscaling has been disabled).
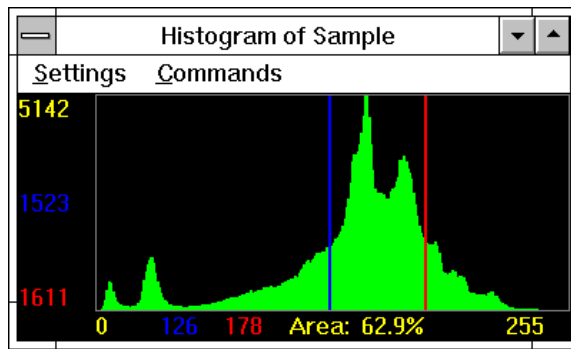
Click the numeric labels and the graph bars in the picture to read a brief description about each item's function.

Configuring the Histogram Settings

Hiding the Histogram Labels

Using Show Highlight Bars

Highlighting Under Bars

Highlighting Between Bars

Turning On Area as Percentage

Turning On Apply LUT to Graph

Scaling Between Bars

Using the Histogram Commands

Stretching an Image's LUT (8-bit)

Scaling an Image (16-bit)

Setting the Histogram's X-Axis

Resetting the Histogram's X-Axis

Using the Histogram Tool

To open and use an image's histogram, use the following procedure:

Step	Action
1	Select the Histogram Tool by clicking it using the left mouse button. The image's histogram will appear.
2	Move the blue or red Highlight Bar (located at the edges of the histogram) to display the grayscale value for the histogram currently below the selected bar. The number of pixels in the image with that grayscale value will also be displayed.
	You can move each bar independently by dragging the desired bar with the left mouse button. To move them simultaneously, hold down the [SHIFT] key while dragging the pointer.

Display Mode Tool (Image Window Toolbar)

The Display Mode Tool allows you to select the Monochrome, Pseudocolor, or a user-defined display mode look-up table (LUT) for an image.

Use this tool to switch between the image's two default look-up tables, Monochrome and Pseudocolor. The Monochrome look-up table is a black and white grayscale display mode. The Pseudocolor look-up table is an arbitrarily assigned color display mode. Or you can use the Configure LUT option in the tool's pop-up menu to configure, save, and apply your own look-up table for use with the image.

Click the *Procedure* button above to select from a list of Display Mode Tool procedures.

Selecting a Display Mode

To select a display mode, use the following procedure:

Step	Action
1	Select the Display Mode Tool by clicking it using the left mouse button.
2	Choose Monochrome or Pseudocolor from the pop-up menu to use one of the standard MetaFluor display modes. The check mark indicates the active display mode. **OR** Select a previously loaded user-defined look-up table from the pop-up menu.
3	MetaFluor will switch to the selected display mode.

Loading an Existing LUT

To load an existing look-up table, such as one of the sample LUTs provided with MetaFluor, use the following procedure:

Step	Action
1	Select the Display Mode Tool by clicking it using the left mouse button. A pop-up menu will appear.
2	Choose Configure LUT. The Define User LUT dialog box will appear.
3	Choose *Load.* The Load LUT File dialog box will appear.
4	Select the icon for the desired file. Use the *Look In* drop-down list box or Up One Level icon button to select the desired folder if necessary.
5	Choose *Open.* The Load LUT File dialog box will close.
6	The newly loaded LUT will appear in the Look-Up Table Mode List Box on the left side of the Define User LUT dialog box. (It will also appear in the Display Mode Tool pop-up menu as a menu item.)
	Refer to **Selecting a Display Mode** for more information about selecting the active look-up table.

Configuring the Display Mode

To configure the display mode using the Configure LUT option, use the following procedure:

Step	Action
1	Select the Display Mode Tool by clicking it using the left mouse button. A pop-up menu will appear.
2	Choose Configure LUT. The Define User LUT dialog box will appear.
3	Select the look-up table that you want to configure from the Look-Up Table Mode List Box on the left side of dialog box.
4	If you want to change the quantization of the image, type the desired value in the *Quantization* text box.
5	If you want to invert a look-up table, select *Invert.*
6	If you want to use a **sawtooth grayscale,** select *Contour.*
7	*Subtract* determines how MetaFluor displays pixels when the Adjust Digital Contrast command causes them to display the value for intensities less than zero.
	When deselected, *Subtract* will direct MetaFluor to clip the gray levels of the pixels and display the value for the intensity zero (this is the default setting).
	Select *Subtract* to "wrap around" the gray values and display the value for the difference of the image window's maximum intensity and the intensity produced by Adjust Digital Contrast.
8	When you have finished configuring the look-up table, you can choose *Save* to save it. The Save LUT File dialog box will appear. Type a file name in the *File Name* text box and choose *Save.*

Defining a User LUT

To define a new look-up table, use the following procedure:

Step	Action
1	Select the Display Mode Tool by clicking it using the left mouse button. A pop-up menu will appear.
2	Choose Configure LUT. The Define User LUT dialog box will appear.
3	Choose *Create.* A new LUT named User0 will appear in the Look-Up Table Mode List Box. *Red, Green, Blue, Upper,* and *Lower* will become available.
4	Choose the < command button on the right side of the dialog box to open the LUT graph. The X-axis values of the graph are the "Input," while the Y-axis values are the "Output."
	If the user LUT is based on a monochrome LUT, only one of the three scan lines will be visible.
5	Select the desired starting and ending look-up table addresses to be modified using *Lower* and *Upper.*
	You can select the entire range or you can select smaller ranges, leaving existing values for other parts of the look-up table intact.
6	The intensity for the red, green, and blue components for the selected range of addresses can be editing by enabling the color component(s) you want to edit using the *Red, Green,* and *Blue* check boxes. You can disable any component you do not want to edit.
	AND For each of the selected components, select a starting value and an ending value using the text boxes next to *Red, Green,* and *Blue.* (If you know the color for the range, rather than the values, you can select the desired color from the *Color* drop-down list and the appropriate values will appear in the starting and ending value text boxes.)
7	Choose *Apply* to apply the edited look-up table to the image.
	(You can repeat Steps 4 - 6 as necessary.)
8	If you want to save the custom look-up table on disk for future use, choose *Save.* Type the desired file name in the *File Name* text box and choose *Save.*

Contrast Tool and Slider (Image Window Toolbar)

The Contrast Tool allows you to autoenhance, reset, or fix the contrast of an image. The Slider, in its default state, allows you to adjust the overall brightness of an image and expand the range of grayscale levels displayed in an image.

Use the Contrast Tool when you want to perform one of the three tasks in the Contrast Tool's pop-up menu: Auto Enhance, Reset Contrast, or Fix Contrast:

Auto Enhance adjusts the contrast by performing a grayscale stretch on only those grayscale levels that are contained in the image's histogram.

Reset Contrast resets the contrast to the default settings of *Contour* = OFF, *Invert* = OFF, *Quantization* = 255, *Brightness* = 50, and *Contrast* = 50.

Fix Contrast changes the pixel gray values in the image permanently, based the image's new LUT values. This feature does not affect 16-bit images.

When the Threshold Tool is in the "Thresholding Off" mode, the slider in the Image Window Toolbar can be used to adjust image brightness and contrast. Dragging with the left mouse button while the Contrast Pointer is over the slider adjusts the overall brightness of the image. Dragging with the right mouse button adjusts the range of grayscale levels displayed, thereby changing contrast. (Contrast can only be *increased*, never decreased, relative to the original image state.)

See Also:

Histogram Tool

Using the Contrast Tool

To use the Contrast Tool, use the following procedure:

Step	Action
1	Select the Contrast Tool by clicking it using the left mouse button.
2	Select one of the following options from the pop-up menu that appears: **Auto Enhance, Reset Contrast,** or **Fix Contrast.**
3	If you choose Fix Contrast for a stack, a dialog box will appear: If you want to fix the contrast for all planes, choose *Yes.* **OR** If you want to fix the contrast for the current plane, choose *No.*

Using the Contrast Slider

To adjust the brightness and contrast with the Contrast Slider, use the following procedure:

Step	Action
1	Position the pointer over the Contrast Slider so that it changes from the Arrow Pointer to the Contrast Pointer.
2	To adjust the brightness of the image, drag the mouse up or down using the LEFT mouse button until you have found the desired contrast setting.
	Note: This will only affect an image's LUT. You must choose **Fix Contrast** if you want to alter the image data permanently.
3	To expand the contrast range, drag the mouse up using the RIGHT mouse button until you have found the desired contrast setting. (You will not be able to decrease the contrast in an image beyond the original state.)
	Note: This will only affect an image's LUT. You must choose **Fix Contrast** if you want to alter the image data permanently.
	Expanding the contrast reduces the number of grayscale levels that you will see in the image at one time (the number of grayscale levels actually available stays the same) but increases the perceived contrast of the image.

Palette Tool (Image Window Toolbar)

The Palette Tool selects the number of grayscale levels used for displaying an image.

Use this tool when you want to change the number of palette entries used for displaying an image. A palette is a fixed number of colors that can be rendered at any one time in the display. The default setting for MetaFluor is *64 Palette Entries.*

The number of images that you can display on the desktop can be increased if each image uses fewer palette entries. However, color images tend to look better with the maximum possible number of palette entries.

The setting for palette entries affects only the number of gray levels or colors used for display; it does not change the bit-depth or actual grayscale values of the image pixels.

Using the Palette Tool

To use the Palette Tool, use the following procedure:

Step	Action
1	Select the Palette Tool by clicking it using the left mouse button. A pop-up menu will appear.
2	Choose one of the following palettes from the menu: *2 Palette Entries,* *16 Palette Entries,* *32 Palette Entries,* *64 Palette Entries,* *128 Palette Entries,* or *236 Palette Entries.* (A check mark will indicate the current palette selection.)
3	MetaFluor will change the palette selection.

Threshold Tool and Slider

Threshold Tool and Slider (Image Window Toolbar)

Creates a boundary between the objects being measured and other parts of an image on the basis of an image's gray levels.

Some measurement commands require that a distinction in the form of a boundary be made between the objects being measured and other parts of an image (referred to as image "segmentation," or "thresholding"). The Threshold Tool allows you to create a boundary on the basis of the image's gray levels. The Threshold Tool displays a menu that provides four choices:

(1) Inclusive, with gray values between the lower and upper threshold limits *included* in the measurement. Pixels with gray values *outside* of the lower and upper threshold limits will be highlighted by a black overlay (and again excluded from measurement).

(2) Exclusive, with gray values between the lower and upper threshold limits *excluded* from measurement. Pixels with gray values *within* this range will be marked in the image with a black overlay.

(3) Off.

(4) Auto Threshold, which applies an inclusive threshold to what MetaFluor perceives to be the objects in the image. This uses a sophisticated algorithm for analysis of a grayscale histogram, measuring the "peaks" at the brighter end of the histogram to select the threshold range. Autothresholding of 24-bit color images uses the Intensity channel.

When thresholding is enabled, the Contrast Slider will change to become the Threshold Slider, and will include a draggable Low Threshold Arrow and High Threshold Arrow. The Threshold Tool button will indicate the current thresholding status. The button displays three bars representing the three states of the Threshold Slider, with a small wedge-shaped arrow under the bar that corresponds to the current state. When thresholding is disabled, the slider will revert to its status as the Contrast Slider, and can be used to adjust the brightness and contrast of the image.

Using the Threshold Tool and Slider

When the Threshold Tool is in one of the "On" modes, the slider in the Image Window Toolbar will change to a Threshold Slider, allowing you quickly to set the threshold range. To use the slider to threshold an image, use the following procedure.

Note: Moving a threshold arrow toward a new value that is past the second threshold arrow will automatically cause both arrows to select the new value.

Step	Action
1	By default, images are initially displayed in Exclusive mode. Select the Threshold Tool by clicking it using the left mouse button. A secondary menu will appear, providing you with four choices:
	Inclusive, Exclusive, Off, and Auto Threshold.
2	Choose one of the options, as appropriate to your needs.
	Note: For 24-bit color images, it is best to use the Auto Threshold mode, rather than the Inclusive or Exclusive modes.
3	If you chose Inclusive in Step 2, the Threshold Tool button will be displayed with the wedge-shaped arrow pointing to the middle bar. If you are thresholding a wavelength image, a black thresholding overlay will cover the entire image, indicating that all pixel values are included for measurement. If you are thresholding a ratio image, the overlay will be red.
	Drag the lower arrow in the slider up to select a lower threshold gray value limit so that the background areas are free of the overlay.
	AND Drag the upper arrow in the slider to select an upper threshold gray value limit that removes the thresholding overlay from any bright background areas.
	Readjust the lower threshold setting if necessary.
4	If the image is in Exclusive mode (the default state), the Threshold Tool button will be displayed with the wedge-shaped arrow pointing to the rightmost bar, and the image will be uniformly covered by the black thresholding overlay (red in ratio images), because the gray level range has not yet been selected.
	Drag the lower arrow in the slider upward to select a lower threshold gray value limit so that

the background is covered with the black
thresholding overlay.

AND
Drag the upper arrow in the slider to select an
upper threshold gray value limit as needed.

5   If you chose Off in Step 2, any previously
     displayed thresholding will be removed.

6   If you chose Auto Threshold in Step 2,
     MetaFluor will perform an analysis of the
     intensity values in the image and then select a
     threshold range automatically.

Using Region Tools

Region Toolbar

Click each tool in the picture of the
Region Toolbar to read a brief description of the tool and to jump to a more detailed
discussion on how to use it.

The Region Toolbar consists of tools to create and manipulate **regions of interest** in an
image. MetaFluor defines a region of interest with a colored region outline, which, when
selected is a dotted, blinking boundary.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace,
or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Click the name of one of the Region Tools in the following list to learn more about its use.
The Region Tools include:

Locator Tool,

Rectangular Region Tool,

Ellipse Region Tool,

Trace Region Tool,

Single Line Tool,

Multi-Line Tool,

Traced Line Tool, and

Auto-Trace Region Tool.

The status bar below the tools indicates which tool is currently active. This area also
displays the coordinates and size of the region that you are creating or editing. For
irregularly shaped regions, a **"bounding rectangle"** is placed over the region, and the
coordinates of its upper left corner are given.

Opening the Region Toolbar

Moving the Region Toolbar

Closing the Region Toolbar

Changing the Color of a Region's Outline

Copying a Region Outline

Shrinking a Region Outline to Fit an Object

Shrinking a Region Outline Using a Three-Button Mouse

 Configuring Region Toolbar Tools

The Down Arrow button next to the status bar opens a pull-down configuration menu which you can use to configure default colors, sizes, and positions for regions, as well the default behavior of the Locator Tool.

Configuring the Default Behavior for Region Tools

Configuring the Default Color for Regions

Locking Region Positions

Locking Region Sizes

Setting Default Region Size

Region Tools

Locator Tool (Region Toolbar)

The Locator Tool is the default tool in the Region Toolbar.

Use this tool to select, move, resize, edit, and delete regions. When you select the Locator Tool, the pointer will look like an arrow.

Click the *Procedure* button above to select from a list of Locator Tool procedures.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Selecting a Region as the Active Region

To select a region as the active region, use the following procedure:

Step	Action
1	Place the pointer inside the desired region.
	If you are selecting a line region, "inside" the region is within five pixels or less from any point on the line.
2	Press the left mouse button.
	The region's outline will change from a solid line to a dotted line to indicate that it is the active region.

Deselecting the Active Region

To deselect the active region, use the following procedure:

Step	Action
1	Place the pointer outside the active region.
2	Press the left mouse button.
	The region's outline will change from a dotted line to a solid line to indicate that it is no longer active.

Editing the Shape of a Region

To edit the shape of a region, use the following procedure:

Step	Action
1	Place the pointer inside the desired region.
2	Double-click the left mouse button.
	Round region handles will appear.
3	To edit the region, drag the individual round handles to the desired locations.

Deleting a Region

To delete a region, use the following procedure:

Step	Action
1	Place the pointer inside the desired region.
2	Press the right mouse button and choose Delete Region from the pop-up context menu that appears. **OR** Hold down the [SHIFT] key and press the right mouse button.
3	The region will be deleted.

Moving the Pointer Using Keystrokes

To move the pointer using keystrokes, press the arrow keys on the keyboard to move the pointer in the desired direction.

Moving a Closed Region

To move a closed region, use the following procedure:

Step	Action
1	Place the pointer inside the desired region.
2	Drag the pointer using the left mouse button.
3	Release the mouse button when the pointer is at the new location. The region outline will move to the location.

Moving a Line Region

To move a line region drawn with any of the line region tools, use the following procedure:

Step	Action
1	With the Locator Tool, click the line region once to make it the active region.
2	Place the pointer over the line.
3	Hold down the left button of your mouse and drag the line to the desired position. Then release the mouse button.

Displaying a Region's X and Y Coordinates, Width, and Height

To display a region's location, use the following procedure:

Step	Action
1	Place the pointer inside the desired region.
2	Press the left mouse button.
	The Region Toolbar status line will display "Region: (left, top)" to indicate the location of the region's top left corner. If the region is elliptical, the coordinates of its central point will be displayed. If the region has an irregular shape, its top left corner will be determined by bounding the object with a rectangular box.

Resizing a Rectangular or Elliptical Region

To resize a rectangular or elliptical region, use the following procedure:

Step	Action
1	Place the pointer above the edge or corner of region's outline.
2	When the pointer changes to a double-headed arrow cursor, drag the region outline. Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor. **Note:** You will be able to resize the region only in the direction indicated by the double arrows. To stretch the region horizontally and vertically simultaneously, the pointer must be over the corner of the region outline so that the double arrows are pointing diagonally.
3	Release the mouse button when the region is the desired size. **Note:** While you are resizing a region, you can determine its size and location by looking at the status line in the Region Toolbar; it will display "Region: (left, top), (width, height)."

Resizing or Reshaping a Line Region

To resize a line region drawn with any of the line region tools, use the following procedure:

Step	Action
1	With the Locator Tool, click the line region once to make it the active region.
2	Place the pointer over the line.
3	Double-click the line. Round handles will appear at the ends of the line region. If the line region consists of more than one segment, nodes will appear at each vertex.
4	Place the pointer over the handle or node that you want to move.
5	Hold down the left button of your mouse and drag the handle or node to the desired position. Then release the mouse button.
6	Repeat Steps 4 and 5 for each handle or node that you wish to move.

Rectangular Region Tool (Region Toolbar)

The Rectangular Region Tool is used to create and manipulate rectangular regions.

Use this tool to create closed regions in which all of the pixels within the boundaries of the region outline are measured. When you select the Rectangular Region Tool, the pointer will change to an arrow cursor with an attached "rectangle."

Click the *Procedure* button above to select from a list of Rectangular Region Tool procedures.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Creating a Rectangular Region

To create a rectangular region, use the following procedure:

Step	Action
1	Select the image window so that it is active, and place the pointer at the desired location.
3	Press the left mouse button. **OR** Press [INS]. A rectangular region will appear.

Creating and Resizing a Rectangular Region

To create and resize a rectangular region, use the following procedure:

Step	Action
1	Place the pointer at the desired location in the active image window.
2	If you want to "lock" the Rectangular Region Tool so as to create a perfectly square region (i.e., X = Y), hold down the [ALT] key.
3	Drag the pointer downward and toward the right using the left mouse button.
4	Release the mouse button when the region is the desired size. A rectangular region will appear.
	Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Deleting Any Region, Made by Any Tool

To delete a region, use the following procedure:

Step	Action
1	Place the pointer inside the region's outline.
2	Press the right mouse button and choose Delete Region from the pop-up context menu that appears. **OR** Hold down the [SHIFT] key and press the right mouse button.
3	The region will be deleted.

Using the Locator Tool Without Switching Tools

To use the Locator Tool without switching tools, use the following procedure:

Step	Action
1	Press and hold the [CTRL] key down while performing the desired Locator Tool task.
	The pointer will change to the arrow pointer used by the Locator Tool to indicate the change in tool status.

Ellipse Region Tool (Region Toolbar)

The Ellipse Region Tool is used to create and manipulate elliptical regions.

Use this tool to create closed regions in which all of the pixels within the boundaries of the region outline are measured. When you select the Ellipse Region Tool, the pointer will change to an arrow cursor with an attached "ellipse."

Click the *Procedure* button above to select from a list of Ellipse Region Tool procedures.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Creating an Elliptical Region

To create an elliptical region, use the following procedure:

Step	Action
1	Select the image window so that it is active and place the pointer at the center of the desired location (ellipses are initially drawn from their center point, rather than the top left corner).
2	Press the left mouse button. **OR** Press [INS]. An elliptical region will appear. **Note:** Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Creating and Resizing an Elliptical Region

To create and resize an elliptical region, use the following procedure:

Step	Action
1	Place the pointer at the desired location in the active image window.
2	If you want to "lock" the Ellipse Region Tool so as to create a perfectly circular region (i.e., X = Y), hold down the [ALT] key.
3	Drag the pointer downward and toward the right using the left mouse button.
4	Release the mouse button when the region is the desired size. An elliptical region will appear.
	Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Single Line Tool (Region Toolbar)

This tool is used to create single line regions.

Use this tool to create line regions that are of a one-pixel width and of any desired length. All measurements will be made on only those pixels that are under the line. When you select the Single Line Tool, the pointer will change to an arrow cursor with an attached line.

Click the *Procedure* button above to select from a list of Single Line Tool procedures.

Starting the Single Line

To start a straight line with the Single Line Tool, use the following procedure:

Step	Action
1	Position the pointer at the desired location.
2	Press the left mouse button.
	The line's starting point will appear, with a rubber band line stretched between the starting point and the current pointer position.

Ending the Single Line

To end the straight line you are drawing with the Single Line Tool, use the following procedure:

Step	Action
1	Move the pointer to the desired ending location.
2	Press the left mouse button.
	A solid, fixed line will appear.

Multi-Line Tool (Region Toolbar)

This tool is used to create lines consisting of more than two or more straight-line segments.

Use this tool to create multiple-point lines that are of a one-pixel width. All measurements will be made on only those pixels that are under the line. When you select the Multi-Line Tool, the pointer will change to an arrow cursor with an attached "multi-point line."

Click the *Procedure* button above to select from a list of Multi-Line Tool procedures.

Starting a Multi-Line Region

To start a line consisting of more than two or more straight-line segments, use the following procedure:

Step	Action
1	Select the image window so that it is active, and place the pointer at the desired location.
2	Press the left mouse button. The line's starting point will appear with a rubber band line stretched between the starting point and the current pointer position.

Completing the First Segment of the Multi-Line

To complete the first segment of a multi-line region, use the following procedure:

Step	Action
1	Move the pointer and attached rubber band line to the next point. (Do NOT hold down the mouse button yet.)
2	Press (and RELEASE) the left mouse button. The rubber band will freely follow the pointer to its new location. A fixed line will appear when the mouse button is clicked.

Adding Additional Segments

To add more segments to the multi-line region, use the following procedure:

Step	Action
1	Move the pointer and attached rubber band line to the next point. (Do NOT hold down the mouse button yet.)
2	Press (and RELEASE) the left mouse button. The rubber band line will freely follow the pointer to its new location. A fixed line attached to the previous segment will appear for each segment you add.
3	Repeat Steps 1 and 2 for each segment you want to add. A fixed line attached to the previous segment will appear for each segment you add.

Ending the Last Line Segment

To end the last segment of the multi-line region, use the following procedure:

Step	Action
1	Move the pointer to the desired location.
2	Double-click the left mouse button.
3	MetaFluor will close the region automatically so that the last point and the first point are joined.

Traced Line Tool (Region Toolbar)

This tool is used to create freehand lines.

Use this tool to make freehand line regions of a one-pixel width. All measurements will be made on only those pixels that are under the line. When you select the Traced Line Tool, the pointer will change to an arrow cursor with an attached "free-hand line."

Click the *Procedure* button above to select from a list of Traced Line Tool procedures.

Starting a Hand-Traced Line

To start a hand-traced line with the Traced Line Tool, use the following procedure:

Step	Action
1	Select the image window so that it is active, and place the pointer at the desired location.
2	Press the left mouse button.

OR
Press [INS].

The region's outline starting point will appear, with a rubber band line stretched between the starting point and the current pointer position.

Adding a Point Using a Straight Line

To draw a straight line to the next point in a hand-traced region with the Trace Region Tool, use the following procedure:

Step	Action
1	Move the pointer and attached rubber band line to the next point. (Do NOT hold down the mouse button yet.)
2	Press (and RELEASE) the left mouse button.
	The rubber band will freely follow the pointer to its new location. A fixed line will appear when you press the mouse button.

Ending the Hand-Traced Line

To end the hand traced line, use the following procedure:

Step	Action
1	Double-click the left mouse button.
2	MetaFluor will end the line.

Auto-Trace Region Tool (Region Toolbar)

The Auto-Trace Region Tool is used to create regions by automatically tracing objects.

Use this tool to create closed regions that delineate objects with well-defined edges. When you select the Auto-Trace Region Tool, the pointer will change to an arrow cursor with an attached "traced region."

Click the *Procedure* button above to select from a list of Auto-Trace Region Tool procedures.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

For best results, you should first configure this tool using the options in its dialog box.

Configuring the Auto-Trace Region Tool

To configure the Auto-Trace Region Tool, use the procedure presented in the following table:

Step	Action
1	Select the Auto-Trace Region Tool. The Auto-Trace dialog box will appear.
2	From the *Edge Detection* group, select *Dark to Bright* if your object is dark and your background is bright. **OR** Select *Bright to Dark* if your object is bright and your background is dark.
3	Select the desired type of edge smoothing from the *Edge Smoothing* group: *None* = no edge smoothing *Average* = based on an average of the edge vertices found by MetaFluor *Median* = based on a median of the edge vertices found by MetaFluor. *Intelligent* = based on determining if a point's value is radically different from those of neighboring vertices, and, if so, replacing that value with one that is close to those of its neighbors.
4	Select a value for *Length* that is greater than the radius of the object (or one-half of the length if it is of rectangular shape). **Note:** It is important that you set the *Length* value correctly. If the value is too large, MetaFluor will include pixels that are not part of the object in its search for edge vertices. If the value is too small, MetaFluor will trace only part of the object, or none of the object if it cannot find edge vertices within the limits of the length value.
5	Select the value for *Angle* to represent the degrees between edge vertices. Select a smaller number for small objects or highly irregular objects.
6	Select a value for *Threshold* that will instruct MetaFluor to consider a difference in gray level of neighboring pixels that is greater than the *Threshold* value to be an edge.
7	Select a value for the *Width* that will instruct MetaFluor to consider only gray level shifts no wider than this value to be an edge.
8	Select *Use Threshold* if you want MetaFluor to use the threshold, set with the Threshold Tool in the Image Window Toolbar, to assist it in

finding the edge.

9   Once you have selected the necessary options for the individual object, you can use the Auto-Trace Tool.

10   To close the dialog box, click the Close button in its upper right corner.

Tracing an Object

To trace an object with the Auto-Trace Region Tool, use the procedure presented in the following table:

Step	Action
1	Select the image window so that it is the active window, and place the pointer over the center of the object.
2	Press the left mouse button. **OR** Press [INS]. A region will appear which closely matches what MetaFluor perceives as the edge of the object. **Note:** Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Trace Region Tool (Region Toolbar)

The Trace Region Tool is used to create and manipulate hand-traced regions of interest.

Use this tool to create hand-traced closed regions in which all of the pixels within the boundaries of the region outline are measured. When you select the Trace Region Tool, the pointer will change to an arrow cursor with an attached "hand-traced region."

Click the *Procedure* button above to select from a list of Trace Region Tool procedures.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Starting a Hand-Traced Region

To start a hand-traced region with the Trace Region Tool, use the following procedure:

Step	Action
1	Select the image window so that it is active, and place the pointer at the desired location.
2	Press the left mouse button. **OR** Press [INS]. The region's outline starting point will appear, with a rubber band line stretched between the starting point and the current pointer position.

Adding Points Using a Freehand Curve

To draw freehand curves with the Trace Region Tool, use the following procedure:

Step	Action
1	After starting the hand-traced region, drag the pointer around the object you want to trace (using the left mouse button).
2	MetaFluor will add a point wherever you drag the pointer while holding down the mouse button.

Deleting the Last Added Point

To delete the last point that was added to a hand-traced region drawn with the Trace Region Tool, use the following procedure:

Step	Action
1	Press the right mouse button. **OR** Press the [BACKSPACE] or [DEL] key.
2	MetaFluor will delete the last added point.

Closing the Hand-Traced Region

To close a hand-traced region drawn with the Trace Region Tool, use the following procedure:

Step	Action
1	Double-click the left mouse button. **OR** Press the [SPACEBAR] key.
2	MetaFluor will close the region automatically so that the last point and the first point are joined. **Note:** Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Using the Region Toolbar

Opening the Region Toolbar

Step	Action
1	From the Graphs menu, choose Define Regions for Measurement. The Select Source Image for Editing dialog box will appear.
2	From the *Image* drop-down list, select the image on which you want to define your regions.
3	Choose *OK.* All other image windows will close temporarily, and the Region Toolbar will appear.

Moving the Region Toolbar

Step	Action
1	Position the pointer over the toolbar title bar.
2	Click and hold the left mouse button while dragging the toolbar to the new location.
3	Release the mouse button when the toolbar is at the desired location.

Closing the Region Toolbar

Step	Action
1	Click the Region Toolbar's Close button in the upper right corner. **OR** Choose *Done Editing Regions* from the Edit Regions dialog box.
2	The Region Toolbar and Edit Regions dialog box will close, and all previously open image windows will reappear.

Changing the Color of a Region's Outline

MetaFluor automatically assigns the color of a
region's outline unless the Use Same Color for New
Regions command is selected (enabled) in the Region
Toolbar configuration menu (the "Down Arrow" button).
However, you can change the color after the region
has been created.

Step	Action
1	Position the pointer over the desired region (or within five pixels, if it is a line).
2	Press the *right* mouse button. A pop-up context menu will appear.
3	Point to the Change Color command. A cascading palette menu of color selections will appear.
4	Choose the desired color from the menu. The current color selection will be indicated with a check mark.
5	When you release the mouse button, the region outline will change to the new color, and the context menu will close.

Copying a Region Outline

You can copy a region outline so that you can use a duplicate outline in another image. MetaFluor will retain the X and Y coordinates of the region so that it can be pasted in the same location as the region in the original image. You can use this command to make a duplicate outline for the same image, but you will need to move it to the desired location.

Step	Action
1	Position the pointer over the desired region (or within five pixels, if it is a line).
2	Press the *right* mouse button. A pop-up context menu will appear.
3	Choose Copy Region from the menu.
4	Select the destination image window for the outline copy so that its title bar is highlighted.
5	Right-click in the image. The context menu will appear.
6	Choose Paste Region from the menu. The copy of the outline will appear.

Shrinking a Region Outline to Fit an Object

You can shrink a region created by the Rectangular, Ellipse, or Trace Region Tools (which create closed regions) to fit an object contained inside the region within a thresholded image. Sections of the region outline that are over background will shrink to find the edge of the object. However, the position for sections of the region outline that are over thresholded pixels will not change. If there isn't an object inside the region, the region outline will not change. If a threshold is not set, this command assumes that any non-zero pixel is object data.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Step	Action
1	Threshold the image if it not a binary image.
2	Select or create a region containing the entire object. Avoid including parts of other objects in the region. The region must be created using the Rectangular, Ellipse, or Trace Region Tool.
3	Position the pointer over the center of the region.
4	Press the *right* mouse button. (The active region tool can be the Locator, Rectangular, Ellipse, or Trace Region Tool.) A pop-up context menu will appear.
5	Choose Shrink to Fit from the context menu.
6	The region outline will shrink to form an outline around the object.

Shrinking a Region Outline Using a Three-Button Mouse

If you have a three-button mouse, you can use a mouse shortcut to shrink a new region created by the Rectangular, Ellipse, or Trace Region Tools to fit an object contained inside the region. This shortcut works just like the Shrink to Fit command and requires a thresholded image. However, the shortcut applies only to newly created regions.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Step	Action
1	Threshold the image if it is not a binary image.
2	Create a region containing the entire object. Avoid including parts of other objects in the region. The region must be created using the Rectangular, Ellipse, or Trace Region Tool.
3	Position the pointer over the center of the region.
4	Press the middle mouse button. (The active region tool must be the Rectangular, Ellipse, or Trace Region Tool.)
5	The region outline will shrink to form an outline around the object.

Configuring the Default Behavior for Region Tools

You can configure the default behavior for tools either to remain active or to revert back to the Locator Tool after creating a region.

Step	Action
1	Click and hold the pointer on the Down Arrow button in the Region Toolbar status bar. A pull-down menu will appear.
2	From the pull-down menu, choose Revert to Locator Tool After Creating Region. A check mark in front of this option will indicate that the current tool will revert to the Locator Tool after a region is created. **OR** If you want a tool to remain active until another tool is selected, deselect Revert to Locator Tool After Creating Region so that the check mark is removed.

Configuring the Default Color for Regions

MetaFluor automatically assigns the color of a region's outline using randomly assigned colors. However, you can change this behavior by specifying that a particular color be used for all new regions.

Step	Action
1	Click and hold the pointer on the Down Arrow button in the Region Toolbar status bar. A pull-down menu will appear.
2	Choose Use Same Color for New Regions from the pull-down menu. A check mark in front of this option will indicate that one color will be used for all new regions. **OR** If you want region colors to be assigned automatically, deselect Use Same Color for New Regions so that the check mark is removed.
3	If you enabled Use Same Color for New Regions, click and hold the pointer on the Down Arrow button again so that the pull-down menu appears.
4	Choose Region Color from the pull-down menu. A secondary menu will appear.
5	Select the desired color from the menu. It will be highlighted with a dark border. The current color for regions will be indicated with a check mark.

Locking Region Positions

Usually, you will want to be able to use the Locator Tool to move regions. However, you can change this behavior by specifying that the current region positions be locked.

Step	Action
1	Click and hold the pointer on the Down Arrow button in the Region Toolbar status bar. A pull-down menu will appear.
2	Choose Lock Region Positions from the pull-down menu. A check mark in front of this option will indicate that the region positions are locked. **OR** If you do not want region positions to be locked, deselect Lock Region Positions so that the check mark is removed.

Locking Region Sizes

Usually, you will want to be able to use the Locator Tool to resize regions. However, you can change this behavior by specifying that the current region sizes be locked. Once region sizes are locked, new regions are drawn based upon the default region size specified with the Set Default Region Size command.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Step	Action
1	Click and hold the pointer on the Down Arrow button in the Region Toolbar status bar. A pull-down menu will appear.
2	Choose Lock Region Sizes from the pull-down menu. A check mark in front of this option will indicate that the region sizes are locked.

OR
If you do not want region sizes to be locked, deselect Lock Region Sizes so that the check mark is removed.

Setting the Default Region Size

The default region size is used to specify the size of rectangular and elliptical regions that are created when you simply click in the image without resizing the region. If Lock Region Sizes is enabled, new regions will be drawn based upon the default region size specified with the Set Default Region Size command.

Note: Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaFluor.

Step	Action
1	Click and hold the pointer on the Down Arrow button in the Region Toolbar status bar. A pull-down menu will appear.
2	Choose Set Default Region Size from the pull-down menu. The Set Default Region Size dialog box will appear.
3	Type the number of pixels for the default horizontal and vertical size in the *X Size* and *Y Size* text boxes, respectively.
4	Choose *Close.*

Graphs

Some MetaFluor commands that display a graph use a standard, non-scrolling graph window. Commands that use graphs with a variable, time-based X-axis, however, typically use open-ended graph windows which are designed to be interactive visual guides to the data currently being acquired.

All graphs in MetaFluor that use a standard, non-scrolling graph window can be configured in a similar manner. You can configure titles, axis ranges and labels, tick marks, trace lines, and element colors. You can also print the graph or copy it to the Clipboard. An example of this type of graph is the one used for adjusting image contrast for an analog video camera.

Commands which acquire and measure data over time, frames, etc., use an open-ended graph window that includes a scroll bar to accommodate the varying size of the X-axis. This type of graph allows you to scale the Y-axis automatically. You can insert event marks with your own text while acquiring the graph data. When you position the pointer over an event mark or trace line, and then press and held down the left mouse button, a status line will be displayed. If you click on an event mark, the event text will be displayed. Otherwise, data from the closest point in the nearest trace line will be displayed. Like the graph trace line, the bullet preceding the displayed data is color-coded to match the color of its region.

TIP: To bring otherwise obscured graph windows to the "top" of your workspace display, choose the Bring Graphs to Front command from the Windows menu, or use its keyboard shortcut, CTRL + G.

Note: The maximum number of measurements that can be displayed and stored in a scrolling graph is 8,000 measurements. When this limit has been reached, measurements on the left side of the graph will disappear as new measurements are made.

Standard Graph Windows

Configuring a Trace Line

Configuring Graph Titles

Configuring the X-Axis or Y-Axis

Configuring the Background

Printing a Graph

Copying a Graph to the Clipboard

Saving a Graph as a Bitmap

Scrolling Graph Windows

Configuring a Trace Line

Autoscaling the Y-Axis

Printing a Graph

Copying a Graph to the Clipboard

Autoscrolling the Graph

Using Shortcuts

Keyboard Shortcuts

Commands that are frequently used in MetaFluor have keyboard shortcuts which activate the command without opening its menu. Keyboard shortcuts are listed on the menu to the right of the command. The keyboard shortcuts are also listed with command descriptions throughout the online Help.

To use a keyboard shortcut, press and hold the first key and then press the second key listed. If it is just one key, simply press the key listed.

EXAMPLE:
The shortcut for Clear Graphs is CTRL + C.
Press and hold the [CTRL] key, and then press the letter [C] on your keyboard. Finally, release the [CTRL] key.

Menu Shortcuts

Underlined letters in a menu command name represent a keyboard alternative to accessing that menu item with a mouse. To open the menu and choose a command without using your mouse, type in sequence:

> [ALT] key, *menu letter*, and *command letter*

EXAMPLE:
To quit MetaFluor, you would type: [ALT], [F], [X].
Press and release the [ALT] key, then the [F] key, and finally the [X] key.

If a menu has two or more commands with the same letter underlined, MetaFluor will select the first command that uses that keyboard letter. Subsequent presses of the same key will select the following commands in the menu that use that letter. Commands are selected by MetaFluor in the order they are listed on the menu.

Intensity Modulated Display (IMD) Mode

The purpose of the IMD display is to display ratio images so that the areas of interest are ratioed while the background areas are blank. The IMD display accomplishes this without the need for thresholding the wavelength image.

Every pixel in the ratio image is generated by ratioing the corresponding pixel of the Wavelength 1 image with that from the Wavelength 2 image (or Wavelength 4 with Wavelength 5). The resulting ratio value is scaled between the minimum and maximum ratio that you expect to obtain, and is then displayed. Typically, a pseudocolor look-up table is used to map colors to the different ratio ranges. For example, if the minimum ratio is set at *0* and the maximum ratio is set at *4*, the bottom half of the pseudocolor display (colors ranging from black and dark blue through cyan and green) will represent ratios between 0 and 2, while the top half of the pseudocolor display (colors ranging from yellow through orange, red, and white) will represent ratios from 2 to 4.

The drawback with the Pseudocolor display is that for pixels with a very low intensity, random noise in the image will have a large effect on the resulting ratio. For example, there may be a background area with intensities that are very close to zero. If imaged by an ideal imaging sensor, the background might read out as gray level 5 on both the Wavelength 1 and the Wavelength 2 image. The ratio for this would be 5/5, which is a ratio of 1. However, with a noisy detector (as is often the case when imaging photon-limited specimens) there will be a certain amount of random noise either increasing or decreasing the intensity of each pixel. In this case, we might have an instance where the same pixel in Wavelength 1 is read as gray level 8 (the actual value of 5 plus 3 noise gray levels) and the corresponding pixel in Wavelength 2 might be read as gray level 2 (the actual value of 5 less 3 noise gray levels). This would give us a ratio of 8/2, or 4. A ratio of 4, using the Pseudocolor scale described above would appear as bright white, while the actual ratio of 1 for this pixel would appear as blue. This large discrepancy is due to the effect of noise on the dim background pixels.

The IMD display accounts for this by using an ingenious technique devised by Dr. Roger Tsien and associates. This technique divides the color scale into a distinct number of color hues. Each color hue is further divided into intensities. For example, a typical division might divide the color scale into eight color hues (purple, blue, cyan, blue-green, green, yellow, orange, and red). Each of these color hues has a range of 32 intensities, ranging from dark to bright. For instance, the green band will range from dark green (essentially black) to bright green.

The IMD display uses the ratio to determine the color hue, but uses the intensity from the wavelength images to determine the intensity of the color hue. In the case initially presented, the ratio of the two pixels would be a *ratio* of 4 and fall into the red hue. However, since the intensity of the wavelengths was so low, the *intensity* of the red would be 0 and the result would be a black pixel. This makes the background disappear from the image.

MetaFluor allows you to choose a tradeoff between color hues for ratios and intensity levels. The number of color hues multiplied by the number of intensities must equal 256. In the previous example, 8 * 32 = 256. You can have other combinations such as 16 hues of 16 intensities each, or 4 hues of 64 intensities each, and so on. You should specify the number of hues that will match the number of different ratios you anticipate seeing. For instance, if all of your data is clustered around a ratio of 1, you would set your minimum and maximum ratio to be very close to *1* and set the number of hues to a low number (such as *2* or *4)* and the number of intensities would be correspondingly large. If you have a dynamic scene with a large number of ratios, you will have to compromise with fewer intensities per ratio.

MetaFluor also allows you to choose which wavelength image will be used to determine the intensity component of the display. You can choose either wavelength image, or you can choose to average the wavelength images and use that averaged value to determine the intensity. Typically, you would choose the brightest image.

The IMD display mode operates independently of the bit-depth (bits per pixel) of the source wavelength images. It depends primarily on the ratio, which is always a floating-point number that will range between the minimum and maximum ratios that you have defined. The intensity component is derived as a percentage of the intensity of the source image. If you have the IMD display set to use 32 intensities, and the intensity component is coming from Wavelength 2, and Wavelength 2 is an 8-bit image (gray levels from 0 to 255), MetaFluor will map gray levels 0 - 7 of the wavelength image as intensity level 0, gray levels 8 - 15 of the wavelength image as intensity level 1, and so on. If the wavelength image is a 16-bit image, MetaFluor will divide the range of intensities of the wavelength image by the number of IMD intensities to find the intensity stepping factor. For example, if the wavelength image went from gray levels 200 to 1000, and the IMD display was set to use 32 intensity levels, gray levels 200 - 224 would map to IMD intensity 0, gray levels 225 - 249 would map to IMD intensity 1, and so on.

If your computer monitor is set to 256 colors, you can use the palette control of the image window (click the " P " button in the Image Window Toolbar) and set the palette entries on the ratio image to 236--the maximum allowed. However, this will cause the other image windows on the desktop to blank out. If you set your computer monitor display to 10-, 12-, or 24-bit color, the ratio and other image windows will be displayed in their best possible format, with no side effects. This configuration is recommended.

List of Available Drop-ins

Drop-in Name	Menu	Description
cond	Run Experiment	Defines sets of experimental conditions that can be used to flag the experiment at appropriate times. Tags logged data.
dac	Analog	Analog measurements option for electrophysiology, calcium or O_2 electrodes, etc.
Dualview	Configure	Splits images acquired with an image splitter into two separate images for two separate wavelengths.
importnd	Utilities	Imports a "multi-dimensional" sequence of images into MetaFluor.
movie	Utilities	Builds and plays movies from images on disk.
save8bit	Utilities	Saves 16-bit Wavelength image files as 8-bit TIFF files.
savecal	Calibration	Saves ratio or calibration scales as *.gry files.
twaincfg	Utilities	Selects a Twain-compliant device for image acquisition and specifies whether to use the device's user interface..

Installing Drop-ins Using the Meta Imaging Series Administrator

To install MetaFluor drop-ins using the Meta Imaging Series Administrator, complete the following procedure:

Step	Action
1	Exit MetaFluor.
2	On your desktop, locate and double-click the Meta Imaging Series 6.0 Icon, then double-click Meta Imaging Series Administrator. **OR** From the start button, locate and open Programs>Meta Imaging Series 6.X >Meta Imaging Series Administrator.
3	After the Meta Imaging Series Administrator dialog box opens, press the F1 key to open the Help file for information about installing and removing drop-ins.

Sensitized Emission

$$FRET = RawFRET - (A * Acceptor) - (B * Donor)$$

Where A and B = Coefficient A and Coefficient B

Sensitized Emission if background subtraction is used

$$(RawFRET - Correction\ Factor)$$

$$- \quad A * (Acceptor - Correction\ Factor)$$

$$- \quad B * (Donor - Correction\ Factor$$

$$= \quad FRET$$

Specified Bleed Through
with Donor and Acceptor

$$RawFRET$$

$$- \quad [Acceptor - (Donor\ in\ Acceptor * Donor)] * [Acceptor\ in\ FRET]$$

$$- \quad [Donor - (Acceptor\ in\ Donor * Acceptor)] * [Donor\ in\ FRET]$$

$$= \quad FRET$$

Specified Bleed Through

if background subtraction is used

$$
\frac{\big[\text{RawFRET} - \text{Correction Factor}\big] - \big[(\text{Acceptor} - \text{Correction Factor}) - (\text{Donor in Acceptor} * (\text{Donor} - \text{Correction Factor}))\big] * \big[\text{Acceptor in FRET}\big] - \big[\text{Donor} - \text{Correction Factor}) - (\text{Acceptor in Donor} * (\text{Acceptor} - \text{Correction Factor}))\big] * \big[\text{Donor in FRET}\big]}{\text{FRET}}
$$

=

Thresholds

Threshold levels are used to separate objects that you want to measure from their background on the basis of differences of gray level. MetaFluor has two active thresholding modes:

In the first mode ("On-Exclusive"), thresholding sets values between a defined set of low and high threshold levels to zero, excluding areas containing pixels with those gray levels or color values from measurement and/or analysis. Excluded areas will be displayed as black. In this mode, only pixels with values below the low threshold or above the high threshold will be measured.

In the second mode ("On-Inclusive"), thresholding sets values below the defined low threshold and above the defined high threshold to zero. In this mode, only pixels with values between the upper and lower threshold levels will be measured. Again, excluded areas will be displayed as black. Typically, the low threshold is adjusted so that the background area is excluded in one or both wavelength images. The high threshold is often used to exclude bright regions, such as cell nuclei. By excluding these portions of the image, it becomes easier to interpret the events of interest that are happening in the specimen.

Note: Thresholding only changes the display of the image and how it is measured; it does not affect actual image gray level information or the data that are saved.

The Monochrome display mode is a black and white grayscale display. The Pseudocolor display contains the same grayscale levels as in monochrome display, but arbitrary colors are assigned to assist you in distinguishing similar grayscale levels.

The brightness and contrast options allow you to adjust the digital contrast of an image. The digital contrast affects the image intensity values that are shown in the image window, not the image data. If you are working with 16-bit images, you should adjust the contrast using the Scale 16-Bit Images command rather than with the digital contrast options.

The brightness option allows you to adjust the overall brightness of an image.

The contrast option allows you to expand the range of grayscale levels displayed for an image. As a result, contrast reduces the number of grayscale levels that you will see in the image at one time (the number of grayscale levels actually available stays the same), but increases the perceived contrast of the image.

Export Log Data - Dialog Box Options

Application

Specifies the application for the DDE link. If several versions of an application are listed, select the one that matches the version of the application you plan to use. The application's default settings for the other options will be displayed when you have selected the desired application. If your application is not listed, select *Other Application* from the drop-down list.

Sheet Name

Specifies the name of the worksheet that you want to use in the DDE-linked spreadsheet program.

Starting Row

Specifies the first row number you want to use for logging data. Must match the numerical or alphabetical format used by the application.

Starting Column

Specifies the first column number you want to use for logging data. Must match the numerical or alphabetical format used by the application.

Application Name

This option appears only if you have selected *Other Application* as the DDE-linked application. This option is defined by the application receiving the data. You will need to contact the application's technical support staff to obtain this information. This entry typically is a single word that refers to the software.
EXAMPLE: EXCEL.

Topic Name

This option appears only if you have selected *Other Application* as the DDE-linked application. This option is defined by the application receiving the data. You will need to contact the spreadsheet program developer's technical support staff to obtain this information. For spreadsheet programs, it is the name of the worksheet in which the data will be placed.
EXAMPLE: SHEET1.

Item Name

This option appears only if you have selected *Other Application* as the DDE-linked application. This option is defined by the application receiving the data and specifies where the data are to be sent. You will need to contact the application's technical support staff to obtain this information. MetaFluor recognizes two special symbols in this text string: "<r>" which is replaced by the current row number/letter and "<c>" which is replaced by the current column number/letter.
EXAMPLE: R<r>C<c> for an application which uses the format of R1C1 or RAC1.

OK

Instructs MetaFluor to open a DDE link to an open spreadsheet application, using the specified worksheet name, starting row, and starting column.

Cancel

Cancels the command.

Default

Resets the text in the *Sheet Name, Starting Row,* and *Starting Column* text boxes to the default settings for the selected application.

MetaFluor uses a device called a *Bounding Rectangle* when keeping track or making measurements of the coordinates of irregularly shaped regions. This is done by placing an imaginary (that is, not actually drawn) rectangle over the region outline. The sides of the rectangle are perfectly vertical and horizontal, and the smallest rectangle possible is used that still circumscribes all of the outermost reaches of the irregular region outline. This Bounding Rectangle is used for handling the irregular region's coordinates by determining the upper, leftmost point of the rectangle, just as though an actual rectangular region was present.

Selecting the Drive

Click the *Look In* or *Save In* drop-down list box (which version you see will depend on whether you are loading or saving a file) at the top of the dialog box to open its drop-down list. If you don't see the desired drive letter and name, drag the scroll box until you see it in the drop-down list. Click anywhere on the drive name so that it is highlighted.

Selecting the File's Directory

If you don't see the icon for the desired file in the currently displayed folder, click the Up One Level icon button, which has the icon of a manila folder with a superimposed arrow pointing up. This will bring you up one level in the directory structure. You can repeat this step, if necessary, and can even use it to select a different drive. If you need to go down a level, find the icon for the pertinent subfolder in the collection of currently displayed icons, and then choose *Open.* The DOS single period (" . ") and double period (". .") can be typed in the *File Name* text box to specify directories that are secondary to the currently selected directory or that are secondary to the current directory's parent directory, respectively. If you need to create a new folder (directory), choose the Create New Folder icon button, which looks like a manila folder with rays of light emanating from it. Then type a name for the new folder in the text box which appears next to the new folder's icon.

Selecting the File Type

List Files of Type:

All Images

⑦

List Files of Type:

Stack (*.stk)

All Images
All Files (*.*)
TIFF (*.tif)
UIC (*.img)
Bitmap (*.bmp)
Stack (*.stk)
MRC-500 (*.pic) ⑧
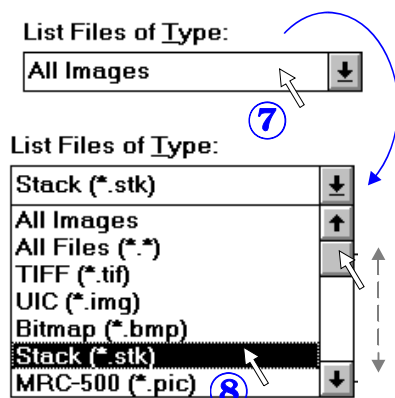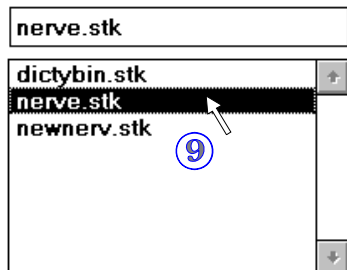
If there many files in the folder, you may want to limit the display of files in the *File Name* list to one particular type. Click the Down Arrow for the *Files of Type* drop-down list box to open its pull-down list. If you don't see the desired file type, drag the scroll box until you see it in the list. Click anywhere on the file type name so that it is highlighted.

When you are saving images, you will need to select a file type. However, for many types of files in MetaFluor, the default file type associated with that kind of file will be selected for you already.

Selecting the File

File **N**ame:

nerve.stk

dictybin.stk
nerve.stk
newnerv.stk

⑨

Click once on the icon for the desired file to display its name in the *File Name* text box.

When you are saving files, you should type a new name in the *File Name* text box, unless you want to select an existing name from the list box and overwrite that file.

Typical Dialog Box Options

Type of Option	Purpose	Selection Procedure
Command buttons	Initiates an immediate action. Dimmed buttons indicate commands that are not currently available.	Choose command button.
Command buttons with ellipsis (. . .)	Opens a secondary dialog box.	Choose command to open next dialog box.
More >> buttons	Expands dialog box.	Choose command button to select from additional options.
Less << buttons	Condenses dialog box to original size.	Choose command button to close extra options.
Text box	Allows user to supply information or choice.	Type requested text at flashing insertion point. Use the [Backspace] or [Del] key to delete text.
List box	Displays a list of choices that doesn't fit into a dialog box.	Click scroll bars with pointer until desired item appears in list. Click an entry to highlight it. You can use cursor keys to advance list until the item Is highlighted.
Drop-down/pull-down lists	Displays only the current selection available in a list. If there are many choices, a scroll bar will be displayed.	Click the arrow at the right of the box to open the list. Select the option in the same manner as a list box.
Radio buttons	Displays mutually exclusive options.	Select the desired option. The circle will fill when it is selected. Selecting a new option clears the

previous selection.

Check boxes	Displayed next to options that are not mutually exclusive.	Select or clear the desired boxes or associated text. Options that are selected will contain a check mark inside the check box.

Configuring a Trace Line

To configure the trace line, use the following procedure:

Step	Action
1	Using the left mouse button, double-click the pointer on or within five pixels of the trace line. The Configure Trace dialog box will appear. Note the sample trace line displayed on the lower right side of the dialog box.
2	Configure the appearance of the trace line using *Trace Style, Mark Style, Line Style,* and *Width:* *Trace Style* enables/disables the connection of trace points from one point to another. *Mark Style* selects the style of markers used. *Line Style* specifies the type of line used. *Width* specifies the pixel width of the line.
3	If you want to change the color of the trace line, select *Change Color.* The Color dialog box will appear. Select a color from the *Basic Color* group and choose *OK.*
4	If you want to change the color of the interior of the graph, select *Set Interior Color.* The Color dialog box will appear. Select a color from the *Basic Color* group and choose *OK.*
5	Use *Range* to set the minimum change in pixel intensity needed before MetaFluor updates a graph for a live or stack image window. Set to *0* for continuous updating. Set to *10* for timely updating with little or no flicker. Avoid large values such as *100.*
6	Select *Apply to All Traces* to apply the same option settings to all traces if more than one trace is available.
7	Choose *OK* when you have finished.

Return to Graphs and Topic Menu

Autoscaling the Y-Axis

To configure MetaFluor to scale the Y-axis range automatically, use the following procedure:

Step	Action
1	Click the Down Arrow in the lower left corner of the graph window to open the pull-down menu.
2	Choose AutoScale Y Axis. A check mark will appear next to the menu entry, and the scrolling graph's Y-axis will be scaled automatically.

Return to Graphs and Topic Menu

Configuring Graph Titles

The graph title and the X-axis and Y-axis titles can be configured using the same configuration procedure. To configure any of the three titles, use the following procedure:

Step	Action
1	Double-click the pointer on the desired title in the graph window using the left mouse button. **OR** Click the Down Arrow in the lower left corner of the graph window to open the pull-down menu. Choose Title, X Title or Y Title from the menu.
2	The Configure Title dialog box will appear.
3	Type a new title name in the *Title* text box.
4	If you want to change the color of the title, choose *Change Color.* The Color dialog box will appear. Select a color from the *Basic Color* group and choose *OK.*
5	Choose *OK* when you have finished.

Return to Graphs and Topic Menu

Configuring the X-Axis or Y-Axis

The graph's X-axis and Y-axis can be configured using the same configuration procedure. To configure either axis, use the following procedure:

Step	Action
1	Double-click the pointer on the desired axis in the graph window using the left mouse button. **OR** Click the Down Arrow in the lower left corner of the graph window to open the pull-down menu. Choose X Axis or Y Axis from the menu.
2	The Configure Axis dialog box will appear.
3	Use *Minimum Value* and *Maximum Value* to select the minimum and maximum gray values to be graphed.
4	Select the number of labeled tick marks along the axis using # *Major Tick Marks.* This number must be divisible into the number of gray levels to be graphed. **AND** Select the number of plain tick marks between the major tick marks using # *Minor Tick Marks.*
5	Select the number of digits of the largest value to be graphed (the maximum value) using # *Digits.*
6	To change the axis color, choose *Change Color.* The Color dialog box will appear. Select a color from the *Basic Color* group and choose *OK.*
7	Choose *OK* when you have finished.

Return to Graphs and Topic Menu

Configuring the Background

To configure the background, use the following procedure:

Step	Action
1	Double-click the pointer anywhere in a corner of the background in the graph window using the left mouse button. **OR** Click the Down Arrow in the lower left corner of the graph window to open the pull-down menu. Choose Background from the menu.
2	The Configure Plot dialog box will appear. Note the sample background displayed on the right side of the dialog box.
3	To change the color of one of the graph's elements, select *Background, Border, Major Ticks,* or *Minor Ticks.* The Color dialog box will appear. Select a color from the *Basic Color* group and choose *OK.*
4	Select the desired format for the graph using the *Plot Format* drop-down list. *Linear* is usually a good choice.
5	Choose *OK* when you have finished.

Return to Graphs and Topic Menu

Printing a Standard Graph

MetaFluor can print a copy of a standard graph to the default printer selected by the Windows Print Manager. To print a copy of a graph, use the following procedure:

Step	Action
1	Click the Down Arrow in the lower left corner of the graph window to open the pull-down menu. Choose Print from the menu.
2	A print message dialog box will appear. Select *Yes* if you want to use a white background with black graphics for printing. **OR** Select *No* if you want to print the graph using its existing background and graphics colors.
3	MetaFluor will display a Print dialog box indicating that it is ready to print the graph to the default printer. Choose *OK* to print the graph.

Return to Graphs and Topic Menu

Printing a Scrolling Graph

MetaFluor can print a copy of a scrolling graph to the default printer selected by the Windows Print Manager. To print a copy of a graph, use the following procedure:

Step	Action
1	Click the Down Arrow in the lower left corner of the graph window to open the pull-down menu. Choose Print from the menu. The Print Setup dialog box will appear.
2	Select the desired printer from the *Printer* group. Choose *OK* when you have finished.
3	MetaFluor will display a Print dialog box indicating that it is ready to print the graph to the default printer. Choose *OK* to print the graph.

Return to Graphs and Topic Menu

Copying a Graph to the Clipboard

MetaFluor can copy a graph to the Clipboard for pasting into other applications. To copy a graph, use the following procedure:

Step	Action
1	Click the Down Arrow in the lower left corner of the graph window to open the pull-down menu. Choose Copy to Clipboard from the menu.
2	MetaFluor will copy the graph window to the Clipboard.
3	Paste the copied graph into the desired application using its Paste command (most programs support the keyboard shortcut, CTRL + V).

Return to Graphs and Topic Menu

Saving a Graph as a Bitmap

MetaFluor can save a graph as a bitmap for use in other applications. The bit-depth of the saved .bmp image will depend on the depth of your video display: if you are using a depth of 8 bits (256 levels) or less, the graph image will be saved as an 8-bit image. If your display has a depth greater than 8 bits, the graph will be saved as a 24-bit image.

To save a graph as a bitmap, use the following procedure:

Step	Action
1	Click the Down Arrow symbol in the lower left corner of the graph window to open the pull-down menu. Choose Save as Bitmap from the menu. The Select Save File dialog box will appear.
2	Type the desired file name in the *File Name* text box. If necessary, use the *Save In* drop-down list box or Up One Level button to change the location for the file.
3	Choose *Save.* MetaFluor will display a message when the graph has been saved. Choose *OK* to confirm the message.

Return to Graphs and Topic Menu

Configuring a Trace Line

To configure the trace line, use the following procedure:

Step	Action
1	Double-click the pointer on or within five pixels of the trace line with the left mouse button.
2	The Set Trace Style dialog box will appear. Note the sample trace line displayed at the bottom of the dialog box.
3	Select the desired pixel width of the trace line using *Line Width.*
4	Select the type of line used to display the trace line using *Line Style.*
5	Select the style of markers used to denote the points on the trace line using *Mark Style.*
6	If you want to change the color of the trace line, choose *Color.* The Color dialog box will appear. Select a color from the *Basic Color* group and choose *OK.*
7	If you want to apply the same option settings to all traces if more than one trace is available, select *Make These Changes to All Graph Traces.*
8	Choose *OK* when you have finished.

Return to Graphs and Topic Menu

Autoscrolling the Graph

A scrolling graph can be set to autoscroll so that the newest data being plotted are always visible. You can enable or disable this feature at any time. To enable or disable the autoscroll feature, use the following procedure:

Step	Action
1	Select the check box (unlabeled) located on the right side of the horizontal scroll bar, so that it is filled in.
2	Start graphing. The graph will automatically scroll so that the most recent data is displayed on the right side of the graph.
3	To disable the autoscroll feature, clear the check box. The graph will only scroll if you scroll it manually using the vertical scroll bar.

Return to Graphs and Topic Menu

Hiding the Histogram Labels

To hide the grayscale and area values displayed in the histogram, use the following procedure:

Step	Action
1	Select the Settings menu from the Histogram menu bar.
2	Choose Labels from the menu so that its check mark is cleared.

Return to Histogram Tool and Topic Menu

Using Show Highlight Bars

When you are working with a binary (1-bit) image, the red and blue Highlight Bars will need to be hidden. The default is for this setting to be enabled so that the highlight bars can be seen while working with 8-bit and 16-bit images.

To hide the red and blue Highlight Bars in the histogram for a binary image, use the following procedure:

Step	Action
1	Select the Settings menu from the Histogram menu bar.
2	Choose Show Highlight Bars from the menu so that its check mark is cleared.
3	The Highlight Bars will be hidden.

Return to Histogram Tool and Topic Menu

Highlighting Under Bars

Highlight Under Bars highlights the pixels in the image which have the gray value selected by the bar in the histogram. For example, if gray level 50 is selected by the blue Highlight Bar, all pixels in the image which have a gray level of 50 will be marked with a blue overlay.

To enable Highlight Under Bars so that you can see the overlays, use the following procedure:

Step	Action
1	Select the Settings menu from the Histogram menu bar.
2	Choose Highlight Under Bars from the menu. A check mark will appear next to the item, indicating that it is enabled.
3	As you slide the red or blue Highlight Bar, the overlay will change to reflect the value selected in the histogram. Red and blue lines will appear in the Contrast Slider to indicate the value that you have selected in the histogram.

Note: If you do not have the palette set to 236 entries, the overlay may "disappear." This is because the limited palette does not include the gray level you selected in the histogram.

Return to Histogram Tool and Topic Menu

Highlighting Between Bars

Highlight Between Bars highlights all pixels in the image that have grayscale values that fall in the range between the blue and red Highlight Bars. Pixels will be marked with a purple overlay. The overlay will extend to those pixels marked by the bars if Highlight Under Bars is disabled. Otherwise, pixels with those precise gray levels will not be marked with the overlay.

To enable Highlight Between Bars, use the following procedure:

Step	Action
1	Select the Settings menu from the Histogram menu bar.
2	Choose Highlight Between Bars from the menu. A check mark will appear next the item, indicating that it is enabled.
3	As you slide the red and blue Highlight Bars, the overlay will change to reflect the values selected in the histogram. A purple line will appear in the Contrast Slider to indicate the values that you have selected in the histogram.

Return to Histogram Tool and Topic Menu

Turning On Area as a Percentage

MetaFluor will display the image area with pixels that have gray levels that fall in the range between the red and blue Highlight Bars. The area can be given either as an absolute number of pixels or as a percentage of total pixels.

To enable Area as Percentage, use the following procedure:

Step	Action
1	Select the Settings menu from the Histogram menu bar.
2	Choose Area as Percentage from the menu. A check mark will appear next to the item, indicating that it is enabled.

Return to Histogram Tool and Topic Menu

Turning On Apply LUT to Graph

Apply LUT to Graph applies the image's look-up table (LUT) when graphing the histogram. This means that commands that change the LUT (such as Stretch LUT or contrast adjustment) will be reflected accurately in the histogram. Apply LUT to Graph should be chosen whenever you adjust the contrast.

Note: This command is intended for use with monochrome images. The grayscale levels from the green channel of the LUT will be those that are graphed using this feature. Since the three channels are identical in a monochrome image, all grayscale levels will be represented in the histogram.

Step	Action
1	Select the Settings menu from the Histogram menu bar.
2	Choose Apply LUT to Graph from the menu. A check mark will appear next to the item, indicating that it is enabled.

Return to Histogram Tool and Topic Menu

Scaling Between Bars

Sometimes a histogram's scaling may not be ideal for your purposes. For example, an image can contain a large number of pixels with gray value 0, causing a tall "spike" in the histogram at gray value 0. To accommodate this "spike" within the Y-axis, the range might become so extended that the grayscale values that you really want to study are not well-represented.

Scale Between Bars rescales the histogram graph so that the grayscale value with the most pixels between the bars becomes the peak gray value. To apply Scale Between Bars, use the following procedure:

Step	Action
1	Move the red and blue Highlight Bars to bracket the gray values of interest.
2	Select the Settings menu from the Histogram menu bar.
3	Choose Scale Between Bars from the menu. A check mark will appear next to the item, indicating that it is enabled.

Note: Rescaling is active only when Scale Between Bars is chosen. If you move the bars while Scale Between Bars is chosen, the scale of the graph will be updated to reflect the new peak gray value. (The Peak Gray Value label at the top of the Y-axis will reflect this change.)

Return to Histogram Tool and Topic Menu

Stretching an Image's LUT

Some images do not have a brightness range that extends through the full range of gray levels available in the histogram display. Stretch LUT allows you to expand the brightness range of the image by selecting a range of grayscale values and "stretching" the actual gray levels to cover the entire range of possible values.

The same number of gray levels missing before the stretch will still be missing, but the values will be spread evenly through the entire histogram. Because this changes the look-up table values but not the actual gray level values, a pixel that is gray level 100 will still be gray level 100 after this command is applied.

Step	Action
1	Move the red and blue Highlight Bars to bracket the gray values of interest.
2	Select the Commands menu from the Histogram menu bar.
3	Choose Stretch LUT.
4	To revert back to the original LUT values, select the **Contrast Tool** and choose Reset Contrast from the pop-up menu that appears.

Return to Histogram Tool and Topic Menu

Scaling 16-Bit Images

The Scale Image command is the same as the **Scale 16-Bit Images** command. A 16-bit image consists of 65536 possible gray levels but, due to experimental conditions, much of your data may reside within a narrow range of gray values. The Scale 16-Bit Images command allows you to scale 16-bit images to a selected range of 256 gray levels, thereby increasing the apparent contrast in the displayed image. This command will not affect measurements; its purpose is to give you control over the brightness and contrast in the image display.

Step	Action
1	Adjust the blue Highlight Bar in the histogram to the darkest gray level you want to use for the images (the same as using *Low Scale* in the Scale 16-Bit Image dialog box).
2	Adjust the red Highlight Bar in the histogram to the brightest gray level you want to use for the images (the same as using *High Scale* in the Scale 16-Bit Image dialog box).
3	Select the Commands menu from the Histogram menu bar.
4	Choose Scale Image. The image will be rescaled.

Return to Histogram Tool and Topic Menu

Setting the Histogram's X-Axis

Set X Axis sets the X-axis scaling of the histogram graph
to the range defined by the blue and red Highlight Bars.
To use Set X Axis, use the following procedure:

Step	Action
1	Move the blue Highlight Bar to the location in the graph that you want to use as the left edge of the rescaled histogram.
2	Move the red Highlight Bar to the location in the graph that you want to use as the right edge of the rescaled histogram.
3	Select the Commands menu from the Histogram menu bar.
4	Choose Set X Axis. The graph will be rescaled.

Return to Histogram Tool and Topic Menu

Resetting the Histogram's X-Axis

Set X Axis sets the X-axis scaling of the histogram graph
to the range defined by the blue and red Highlight Bars.
To use Set X Axis, use the following procedure:

Step	Action
1	Select the Commands menu from the Histogram menu bar.
2	Choose Reset X Axis. The graph's X-axis scaling will be restored.

Invert

Inverting a look-up table remaps the addresses in a look-up
table so that the lowest and highest gray values are switched
as shown in the following table.

When you invert the look-up table of an image containing a
dark background and bright objects, the background will
become bright and the objects will be dark.

Value in Original Table	Value in Inverted Table
0	255
1	254
2	253
3	252
4	251
...	...
251	4
252	3
253	2
254	1
255	0

Using the Contrast Tool and the Contrast Slider

Auto Enhance allows MetaFluor to adjust the contrast by performing
a stretch on only those grayscale levels that are contained in the image's histogram.

Reset Contrast resets the contrast to the default settings of
Contour = OFF, *Invert* = OFF, *Quantization* = 255, *Brightness* = 50,
and *Contrast* = 50.

Adding a Point Using a Straight Line

To add a straight-line segment to a hand-traced line, use the following procedure:

Step	Action
1	Move the pointer and attached rubber band line to the next point. (Do NOT hold down the mouse button yet.)
2	Press (and RELEASE) the left mouse button.
	The rubber band line will follow the pointer to its new location. A fixed line will appear when you press the mouse button.

Adding Points Using a Freehand Curve

To continue a hand-traced line with a freehand curve, use the following procedure:

Step	Action
1	After starting the hand-traced line, drag the pointer around the object that you want to trace (using the left mouse button).
2	MetaFluor will add a point wherever you drag the pointer.

Deleting the Last Added Point

To delete the last point added to a hand-traced line, use the following procedure:

Step	Action
1	Press the right mouse button. **OR** Press the [BACKSPACE] or [DEL] key.
2	MetaFluor will delete the last added point.

Locator Tool

This is the default tool in the Region Toolbar. It is used
to select, move, resize, edit, and delete regions.

Rectangular Region Tool

This tool is used to create and manipulate rectangular regions.
Unlike a line tool, the regions that this tool creates are closed regions
in which all of the pixels within the boundaries of the region outline
are measured. When the Rectangular Region Tool is selected,
the pointer will change to an arrow with an attached rectangle.

Ellipse Region Tool

This tool is used to create and manipulate elliptical regions.
Unlike a line tool, the regions that this tool creates are closed regions
in which all of the pixels within the boundaries of the region outline
are measured. When the Ellipse Region Tool is selected, the pointer
will change to an arrow with an attached ellipse.

Trace Region Tool

This tool is used to create and manipulate hand-traced regions of interest.
Unlike a line tool, the regions that this tool creates are closed regions
in which all of the pixels within the boundaries of the region outline
are measured. When the Trace Region Tool is selected, the pointer
will change to an arrow with an attached hand-traced region.

Single Line Tool

This tool is used to create single lines. The regions that this tool makes
are of a one-pixel width, and all measurements will be made on only
those pixels that are under the line. When the Single Line Tool is selected,
the pointer will change to an arrow with an attached line.

Multi-Line Tool

This tool is used to create multiple-point lines. The regions that this tool makes
are of a one-pixel width, and all measurements will be made on only those pixels
that are under the line. When the Multi-Line Tool is selected, the pointer will change
to an arrow with an attached multi-point line.

Traced Line Tool

This tool is used to create freehand lines. The regions that this tool makes
are of a one-pixel width, and all measurements will be made on only those
pixels that are under the line. When the Traced Line Tool is selected, the pointer
will change to an arrow with an attached free-hand line.

Auto-Trace Region Tool

This tool is used to create regions by automatically tracing objects.
It works best on objects which are clearly singular objects with
well-defined edges. When the Auto-Trace Region Tool is selected,
the pointer will change to an arrow with an attached traced region.

A section of an image designated for image operations.
All such operations occur within a region when it is selected.
Also referred to as an "ROI."

Preparing for Image Acquisition

To prepare for image acquisition, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel dialog box will appear.
2	Focus the microscope using the *Focus* command.
3	If you want to use background subtraction and/or shading correction, acquire the appropriate reference images and enable *Subtract Backgrounds* and *Shading Correction* using the **Reference Images** command.
4	Configure the desired wavelength, binning, exposure time, acquisition region options using the **Configure Acquisition** command.
5	If you want to save images, ratios, or log data, open the appropriate file using the **Open Save Images File, Open Save Ratios File,** or **Open Measurements File** commands. **Note:** The appropriate command will be opened automatically if *Save Images, Save Ratios,* or *Log Data* is selected but a file has not yet been opened.
6	If you plan to log data, you may want to enable *Log Data* in the Experiment Control Panel now so that the location, size, and area of the regions are logged at the start of the log file. Do not select *Save Images* or *Save Ratios* in the Experiment Control Panel yet.
7	Use the **Define Regions for Measurement** command to define the desired regions of interest (necessary for measurements).
8	From the Experiment Control Panel, choose *Set Timelapse.* The Set Timelapse dialog box will appear. Select the timelapse interval and the measurement units using *Timelapse Interval.* Select *0* for no timelapse. Select the number of acquisitions to acquire using *Number of Acquisitions.* Select *0* if you want the acquisition to continue until you choose *Pause Acquisition.* Choose *Close* to return to the Experiment Control Panel.

Running the Experiment

To acquire images and/or data, use the following procedure:

Step	Action
1	From the Run Experiment menu, choose Experiment Control Panel. The Experiment Control Panel will appear.
2	Choose *F4: Acquire* or press the [F4] function key. MetaFluor will start image acquisition.
3	Whenever you want to save ratios or images, select *Save Images* or *Save Ratios* to enable saving. Clear the *Save Images* or *Save Ratios check boxes* when you want to stop saving wavelength or ratio images. EXAMPLE: You can enable and disable *Save Images* so that images are saved from cycles 3 - 10 but not save images from cycles 1, 2, and 11.
4	You can enable or disable *Log Data* at any point during acquisition.
5	Mark events or move regions as necessary during the experiment.
6	The status line will report "Acquiring Wavelength *X*," "Transmitting Wavelength *X*," "Ratioing images," or "Next acquire in XX ms." If the acquisition time selected is shorter than the time required to complete acquisition tasks, the next acquisition will start after the previous one was finished.
7	Press the [F2] key on the keyboard or choose *F2: Pause* to stop the acquisition at any time.

Histogram of Scaled 16-Bit Image
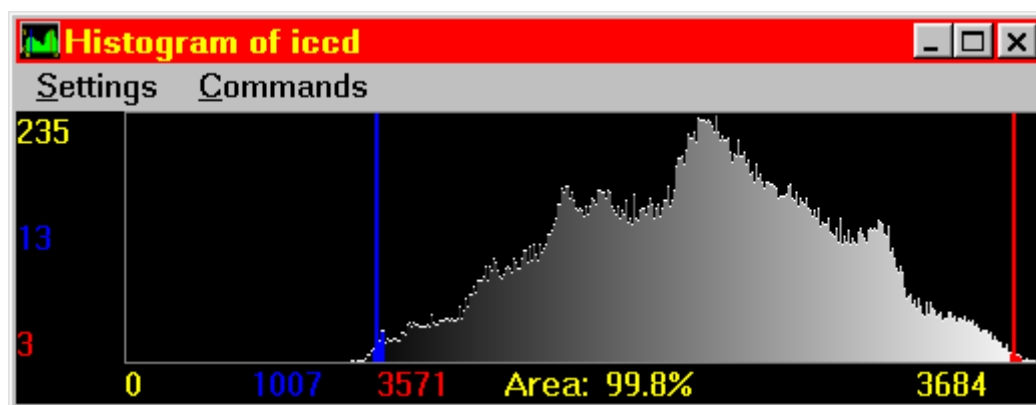
The darkest and brightest 0.1% of the pixels in the image are excluded, and the scaling will be based on the values of the lowest and highest remaining grayscale values.

Scaling Example

Consider a CCD camera with a 12-bit chip. It generates images with intensity ranges that can fall anywhere between gray levels 0 and 4096. If you scaled the entire range of the chip, you would take the range, which would be (4096 - 0) = 4096, and divide that by 256. This results in 16 *data* intensity levels per *displayed* intensity level. This means that each span of 16 gray levels coming from the chip would be represented on the monitor by a single gray level. Thus, gray levels 0 - 15 on the chip would appear as gray level 0 (black) on the monitor, levels 16 - 31 on chip would be gray level 1 on the monitor, and so on up to gray levels 4080 - 4095 on the chip, which are displayed as gray level 255 (white) on the monitor.

Usually, however, the entire dynamic range of the chip is not represented in the acquired data. For example, the camera may have a bias level of around 70, which means that no pixel would have a grayscale value less than 70. You may be imaging a faint signal whose brightest value is 500. If we used 70 as the start of the scaling and 500 as the end of the scaling, the range would be 500 - 70 = 430 levels, and dividing this by 256 results in 1.7 data intensity levels per displayed intensity level. Thus, every 1.7 gray levels on the chip, starting from gray level 70, would be represented by a different intensity level on the computer screen. Gray levels 70 and 71 would be 0 (black) on the monitor, level 72 would be gray level 1, levels 73 and 74 would be gray level 2, and so on, up to gray levels 498 and 499, shown as gray level 255 (white) on the monitor.

Autoscaling finds the darkest and the brightest pixels in the image. These values are then used as the scaling values. Autoscaling lets you see the full dynamic range of the 16-bit image, with equal contrast for all of its gray shades.

However, if you are acquiring two or more wavelengths, you may need to compare the wavelength images visually. If you want to do so, you should not use *Autoscale All.* This is because one image may have very dim gray levels, and the other(s) may have very bright gray levels, but they will look the same after they have each been independently autoscaled.

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